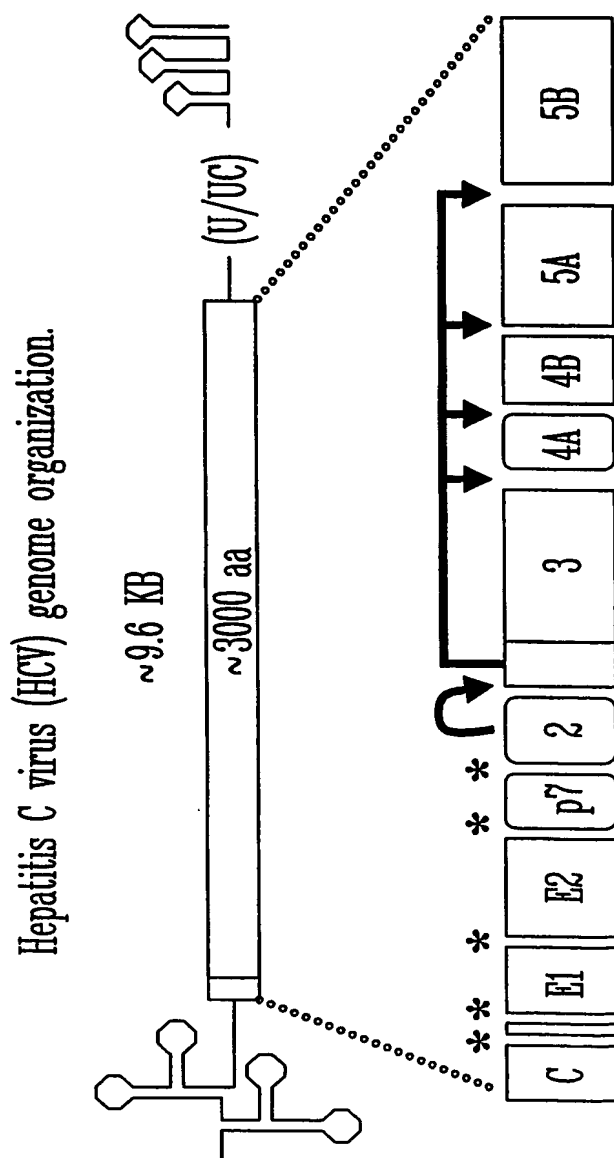


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五

Hypothetical model of the HCV replication cycle

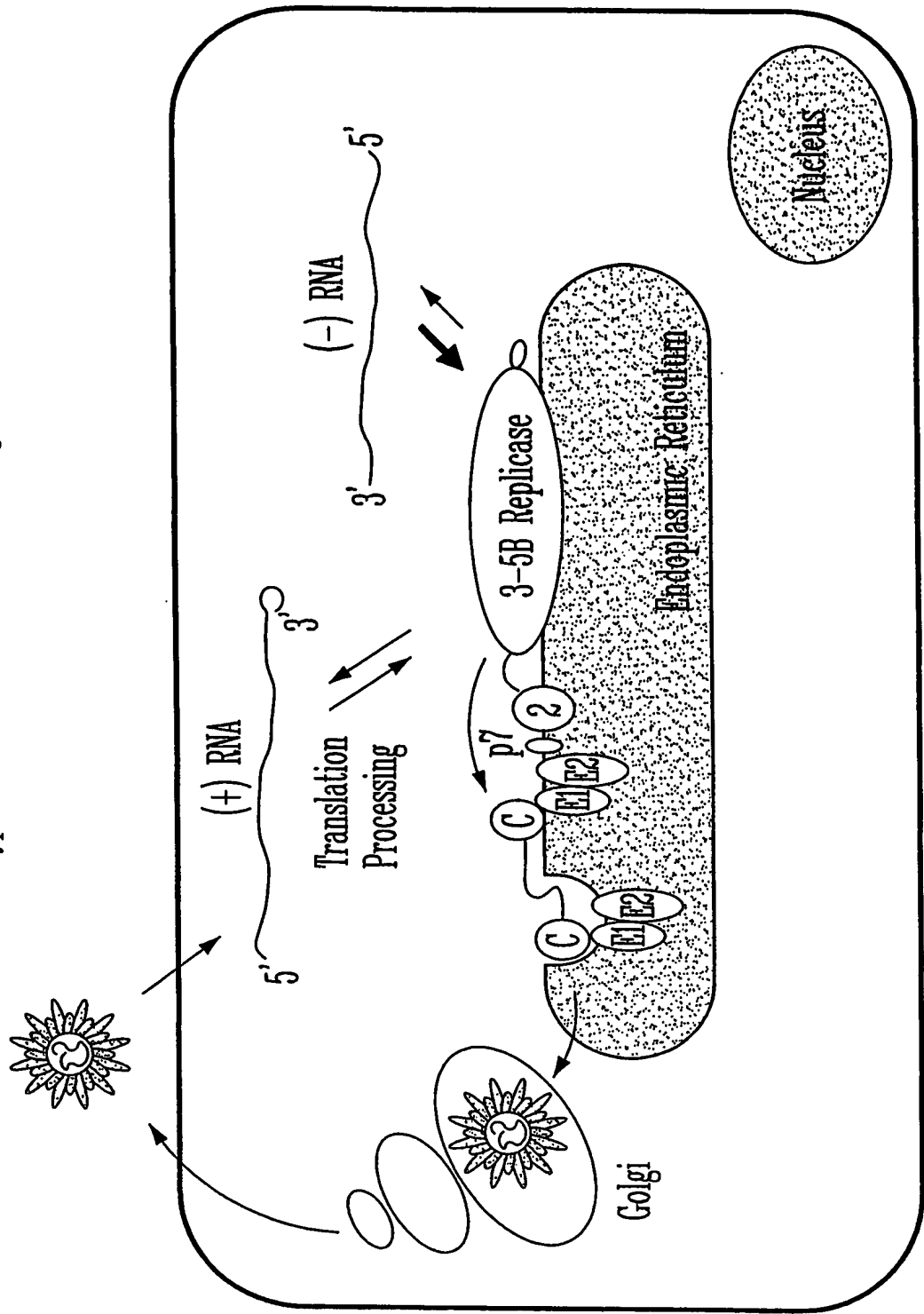


FIG. 2

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Experimental Protocol.

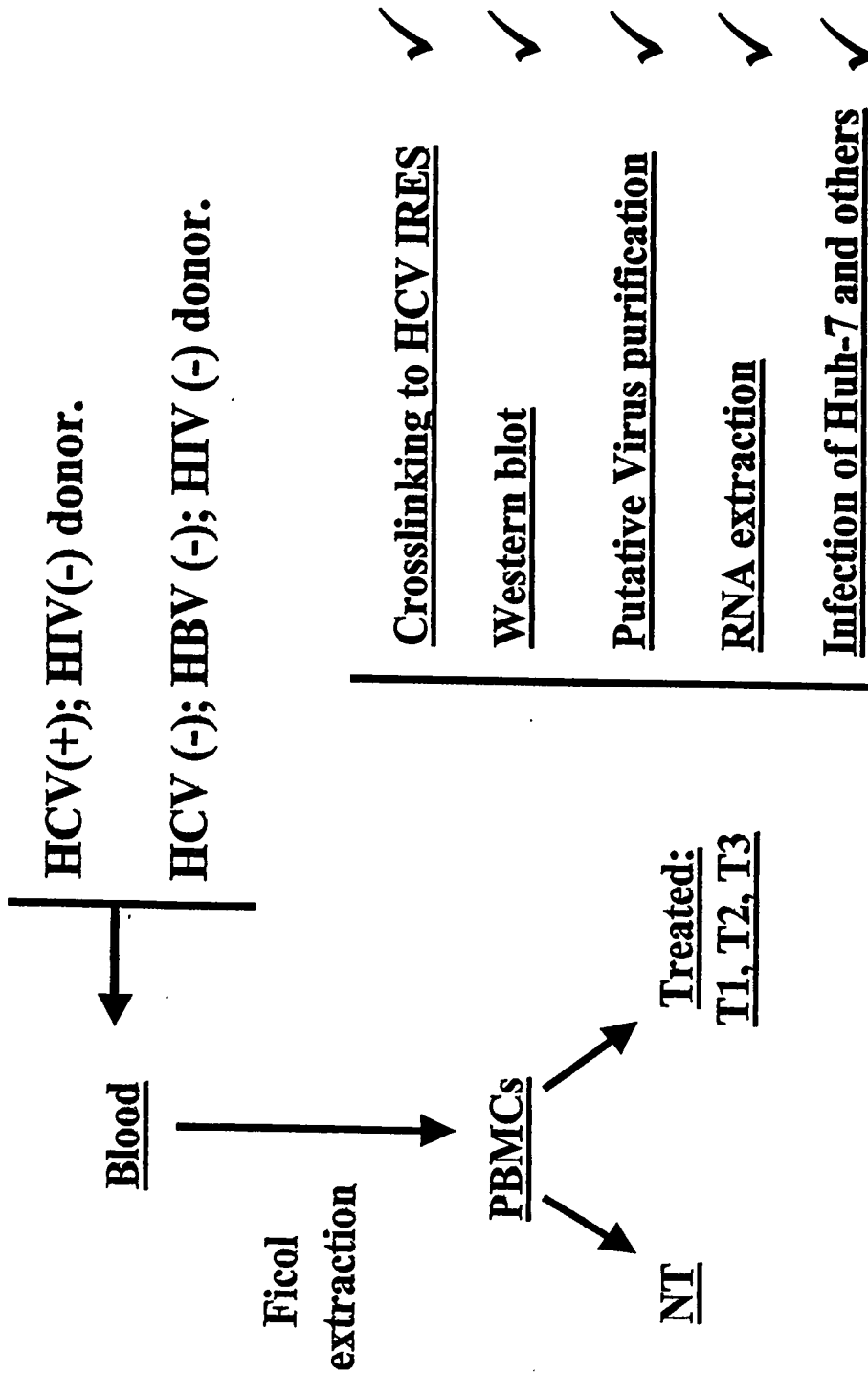
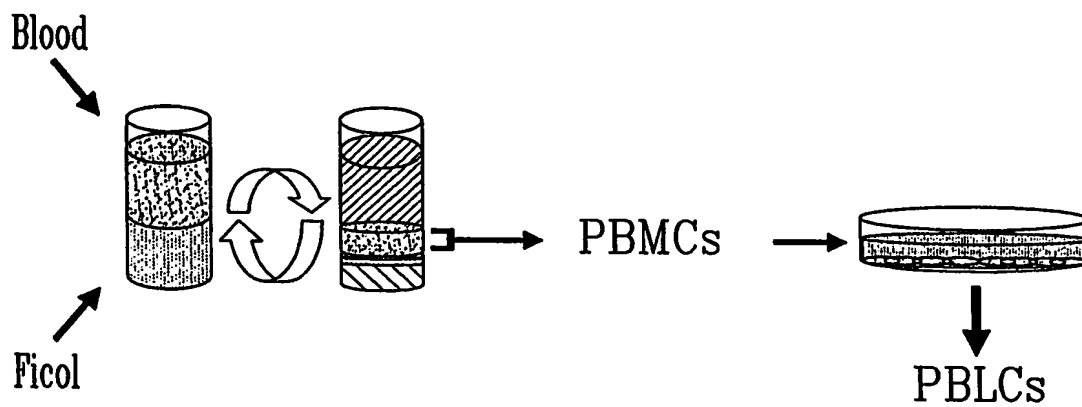


FIG. 3

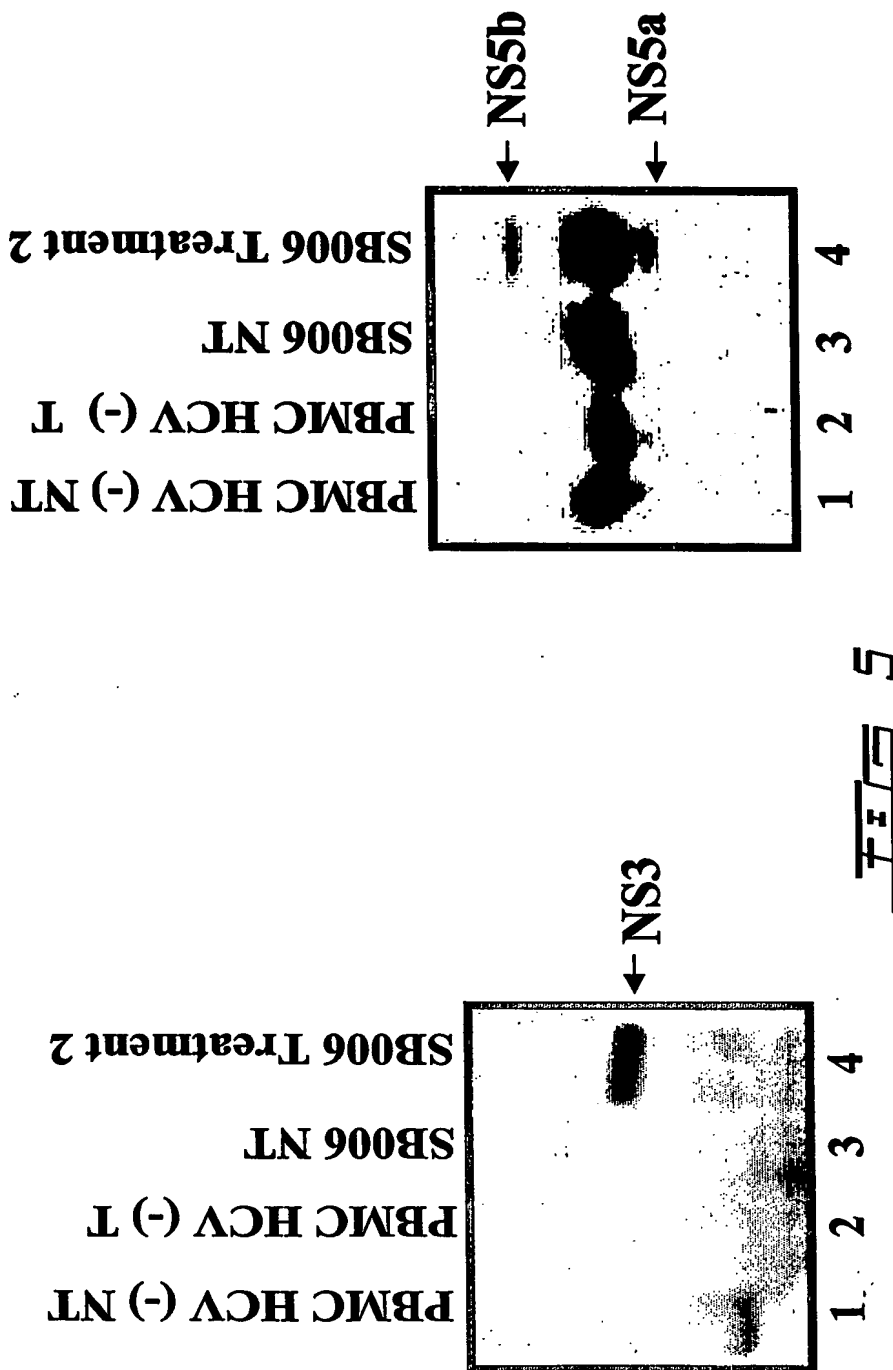
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PBMC and PBLc purification from blood samples.

FIG. 4

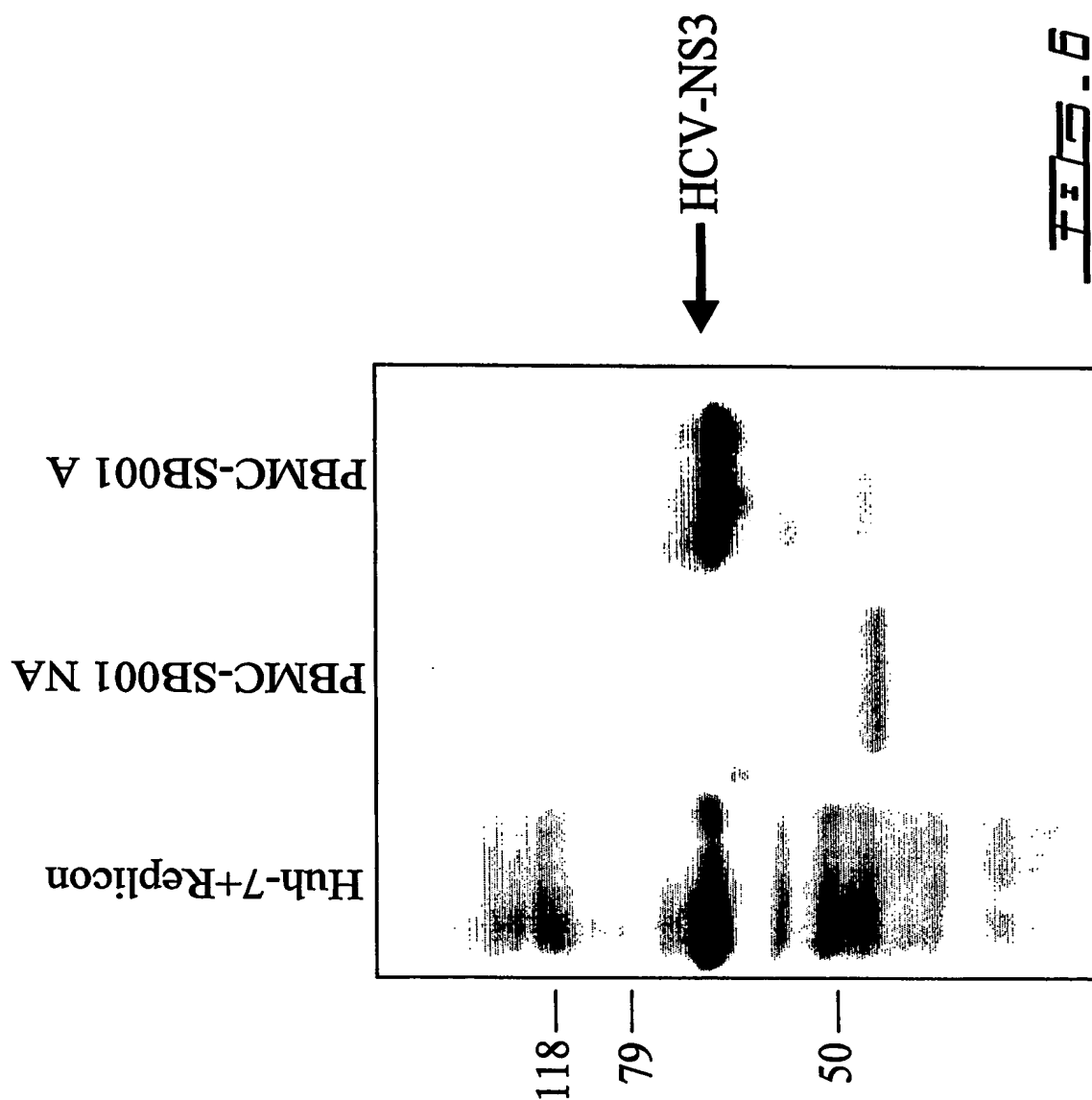
Detection of HCV NS3 and NS5 proteins in cell extracts from Treated

PBMC from an HCV (+) patient.
[Boeringeranti-NS3 polyclonal antibody]

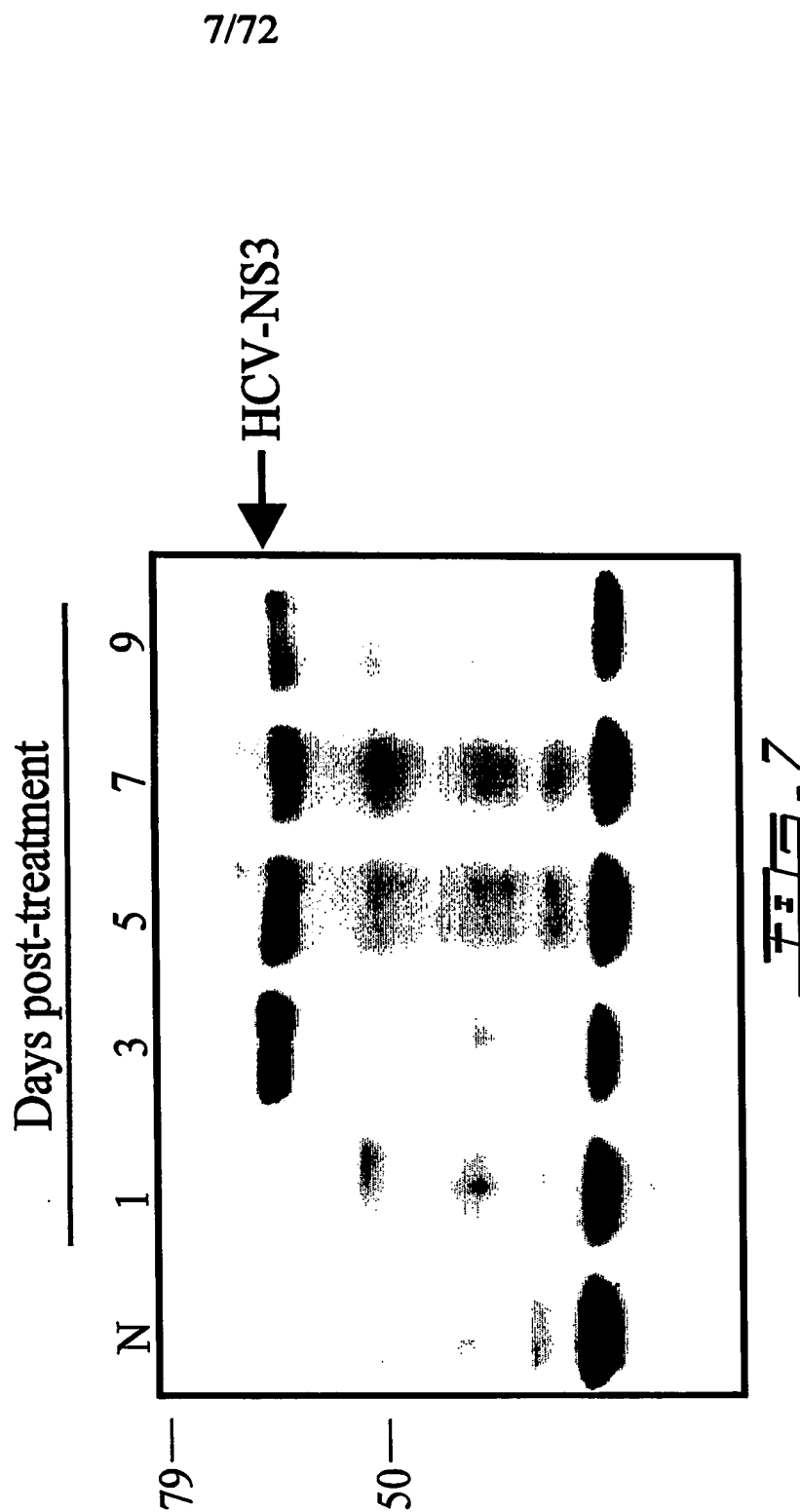


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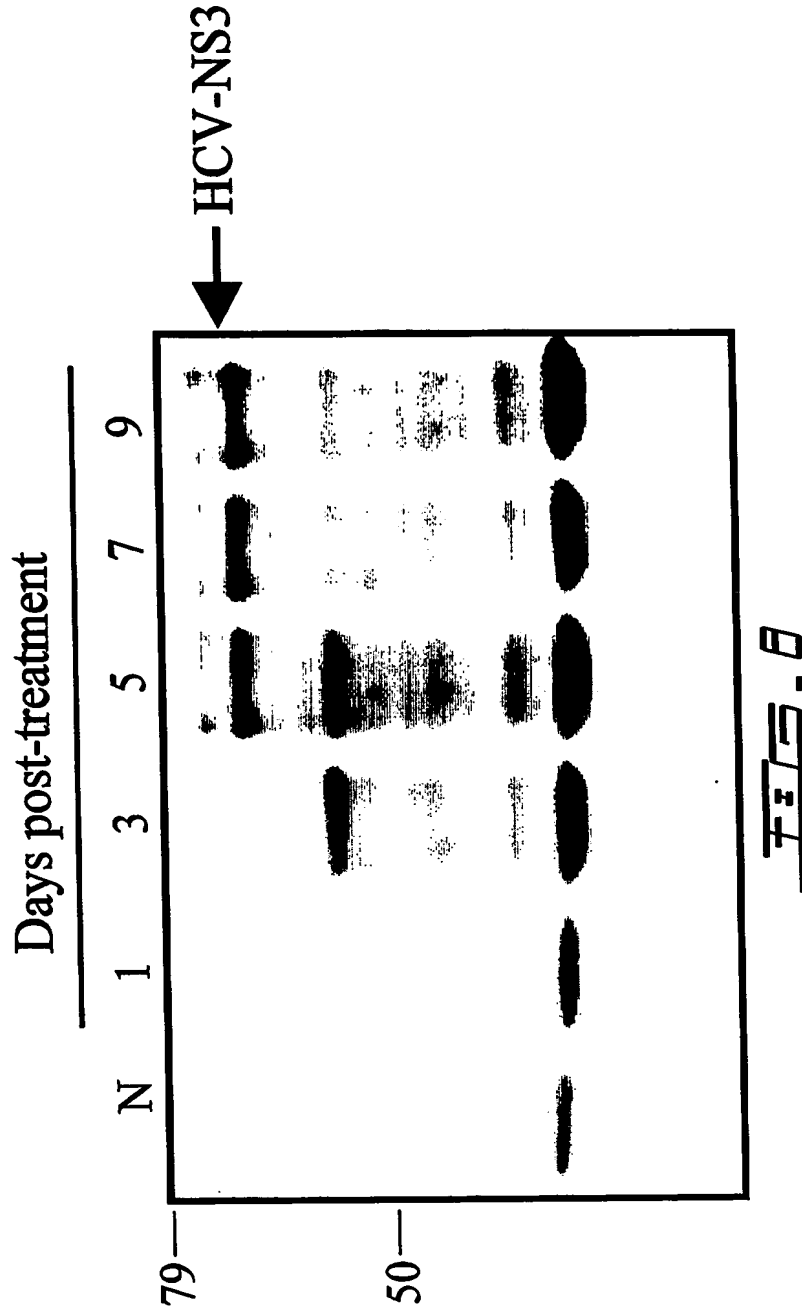


Time course of HCV-NS3 detection: PBMCs From patient MLL-001



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**Time course HCV-NS3 detection:
PBMCs from patient MLL-002**



Detection of HCV-NS3 protein in treated (N3) PBMCs from HCV9+) donors

Huh-7
Huh-7+ replicon

PBMCs
HCV (-) donor.

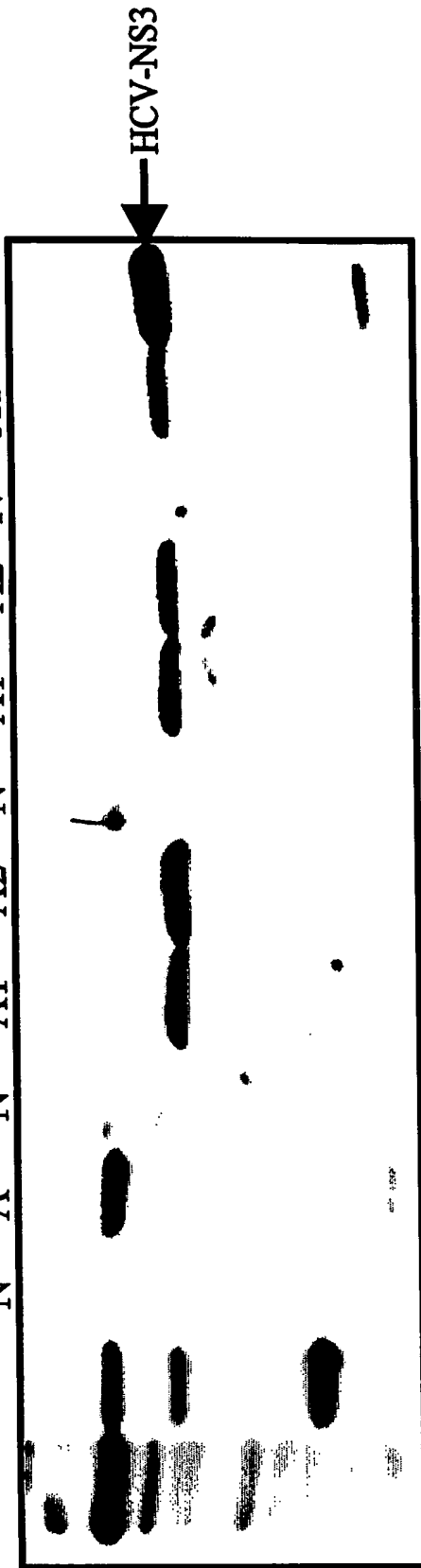
PBMCs
SB006

PBMCs
SB004

PBMCs
SB005

N A N A1 A2 N A1 A2 N A1 A2

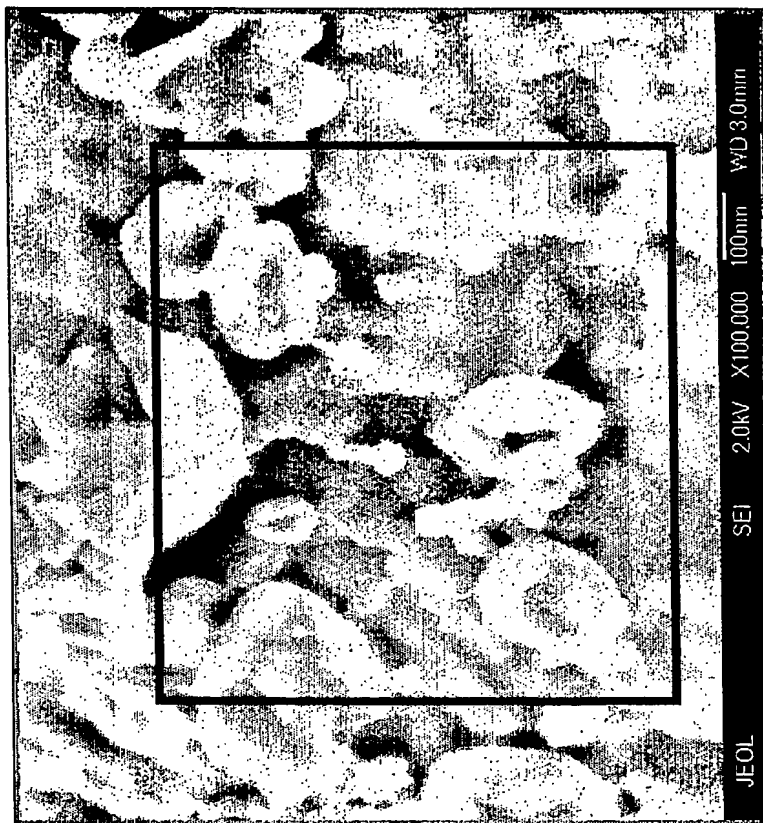
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7-13-9

Detection of virus like particles by scanning electron microscopy

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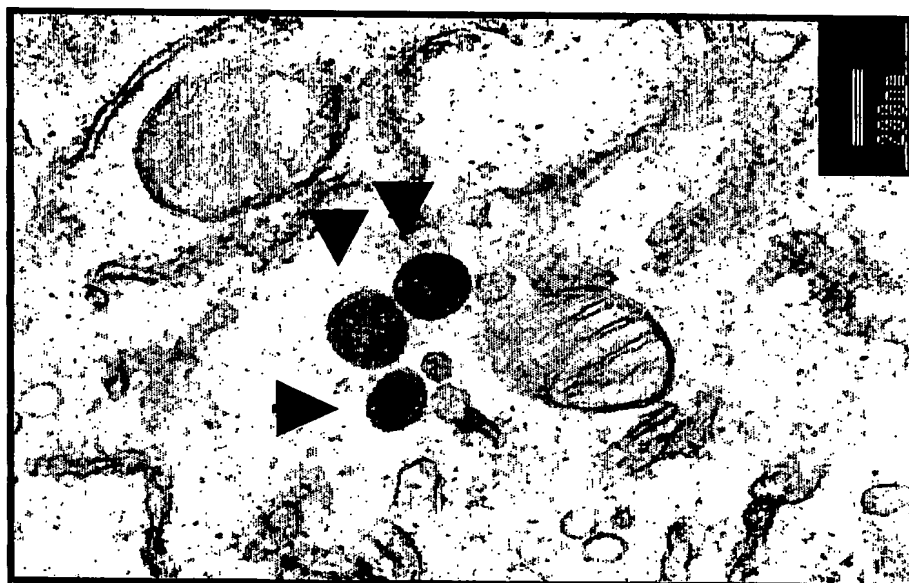


(-) Control

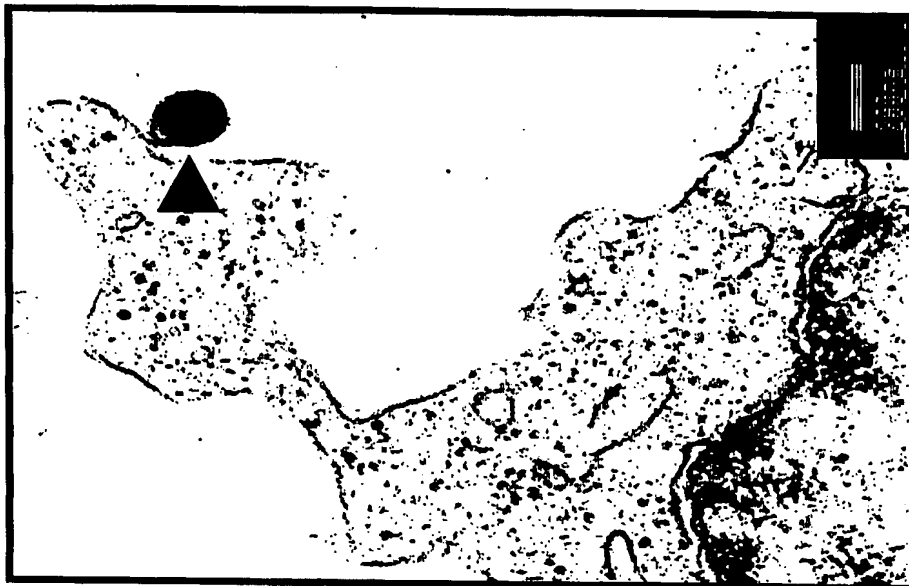
FIG. 10

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**Electron microscopy of Activated PBLs;
Detection of virus like particles**



200 nm

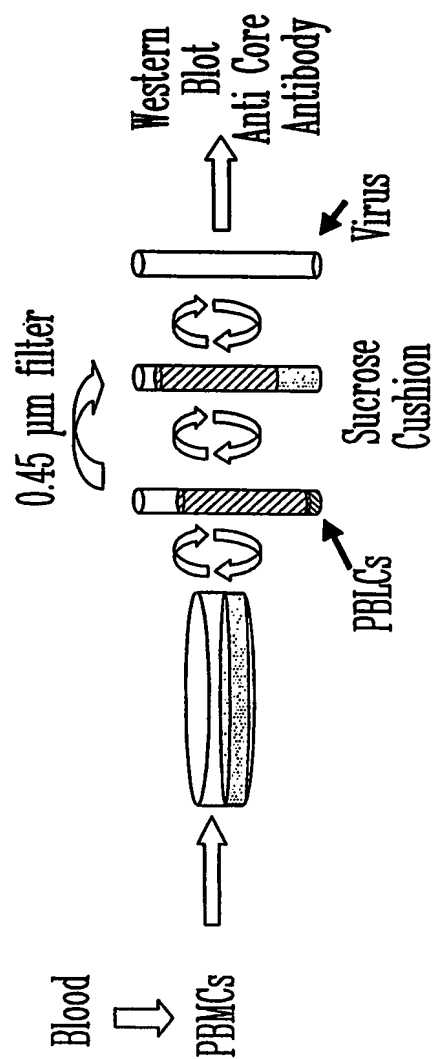


200 nm

FIG. 11

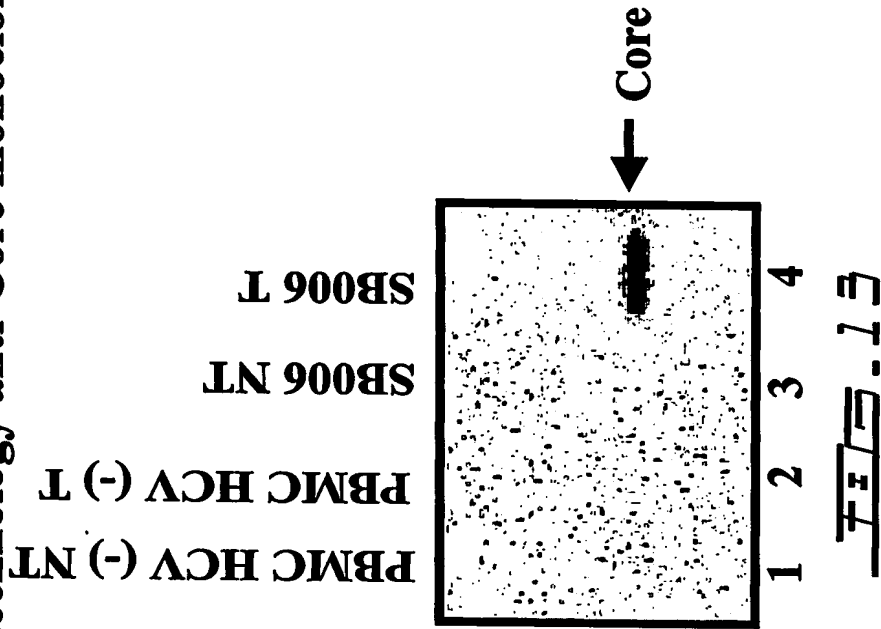
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Virus partial purification.

FIG. 12

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**Detection of HCV Core protein in supernatant of treated
PBMC from an HCV (+) patient.
[Maine biotechnology anti-Core monoclonal antibody]**



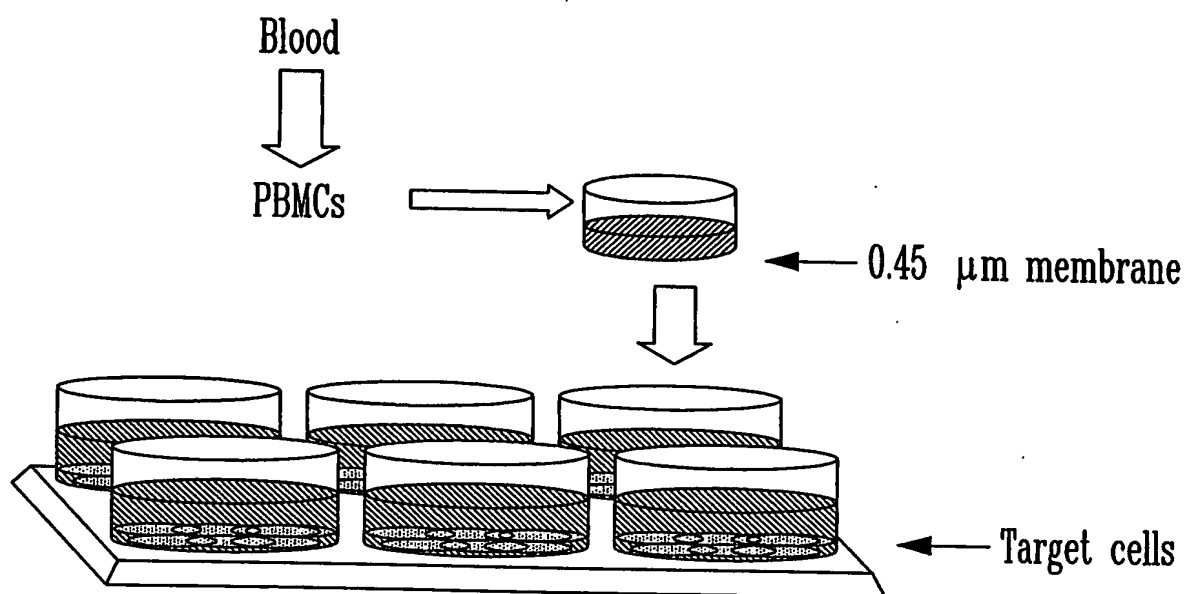
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RNA Quantification I (virus copies/ng total RNA)

Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant
<u>After 4 days</u>		
SB004 NT	2x10 ³	No
SB004 T	2x10 ³	Yes
SB006 NT	1.8 x10 ³	No
SB006 T	2x10 ²	Yes
<u>After 20 days</u>		
SB004	0.00	
SB006	0.00	FEF - 14

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Infection assay; co-culture

FIG. 15

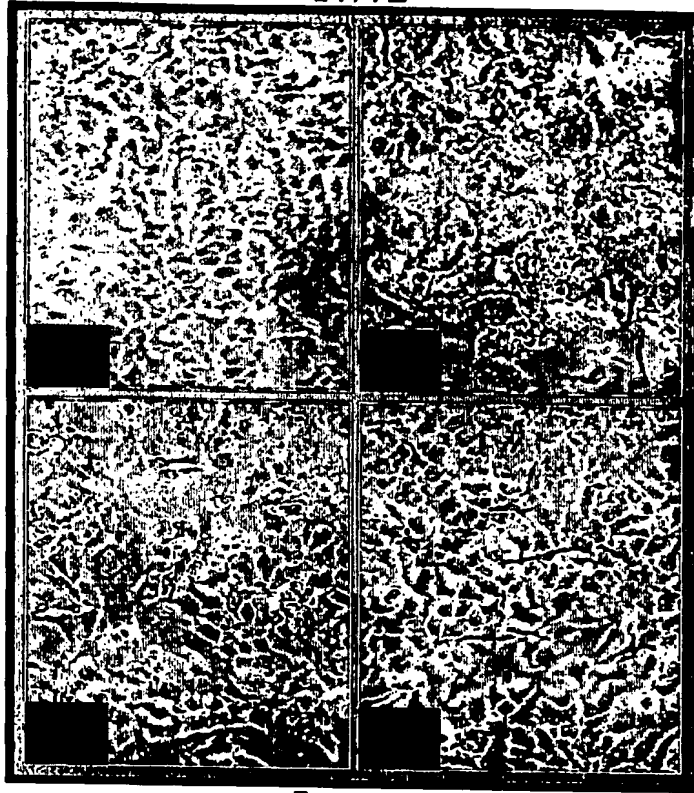
Infection of MT-4 cells
RNA Quantification II (virus copies/ng total RNA)

Patient	HCV RNA In PBMC	Detection of Core (wb) in supernatant	HCV RNA In MT-4
<u>After 10 days</u>			
SB001 NT	13	No	0.00
SB001 T	12	Yes	1600
<u>After 20 days</u>			
SB001	0.00		0.00
SB001	0.00		0.00
		<u>YES - 15</u>	

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Co-culture of Huh-7 and HCV (-) PBMCs.

- 1- Huh-7
- 2- Huh-7 + PBMCs HCV (-) NT
- 3- Huh-7 + Treatment
- 4- Huh-7 + PBMCs HCV (-) T

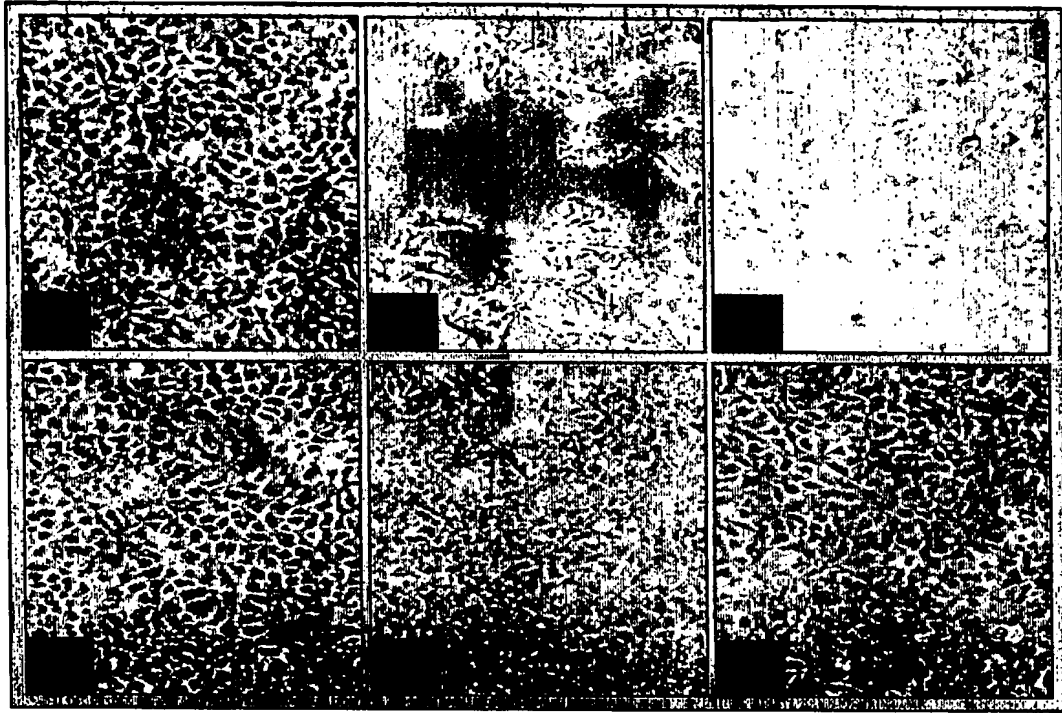


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FIG. 17

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Co-culture of Huh-7 and HCV (+) PBMS° Cs (SB0006).

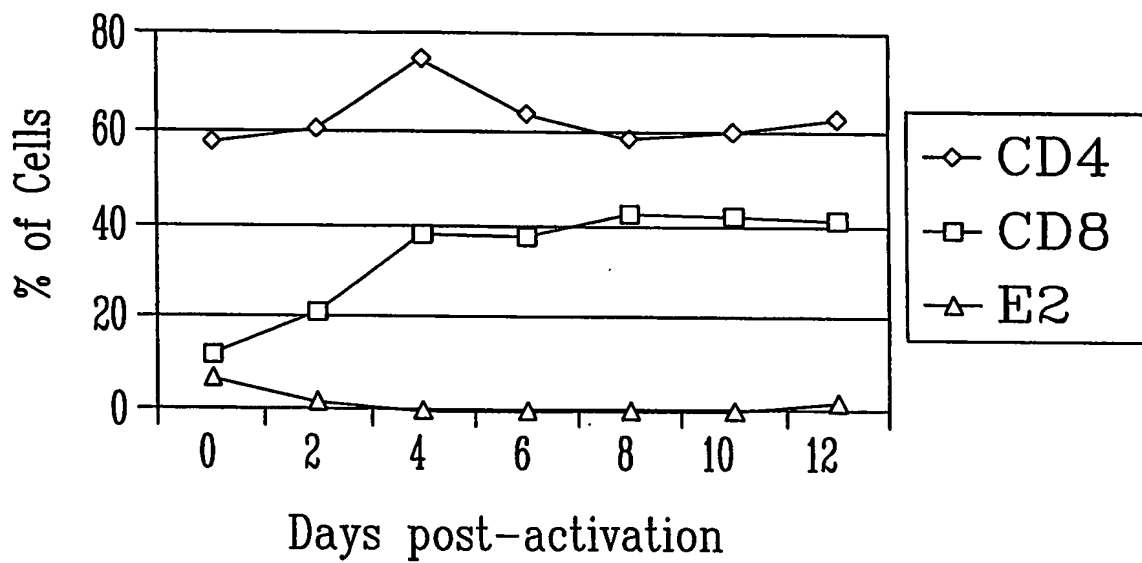


1. Huh-7
- 2-3. Huh-7 + PBMCs HCV (+) NT
4. Huh-7 + Treatment
- 5-6. Huh-7 + PBMCs HCV (+) T

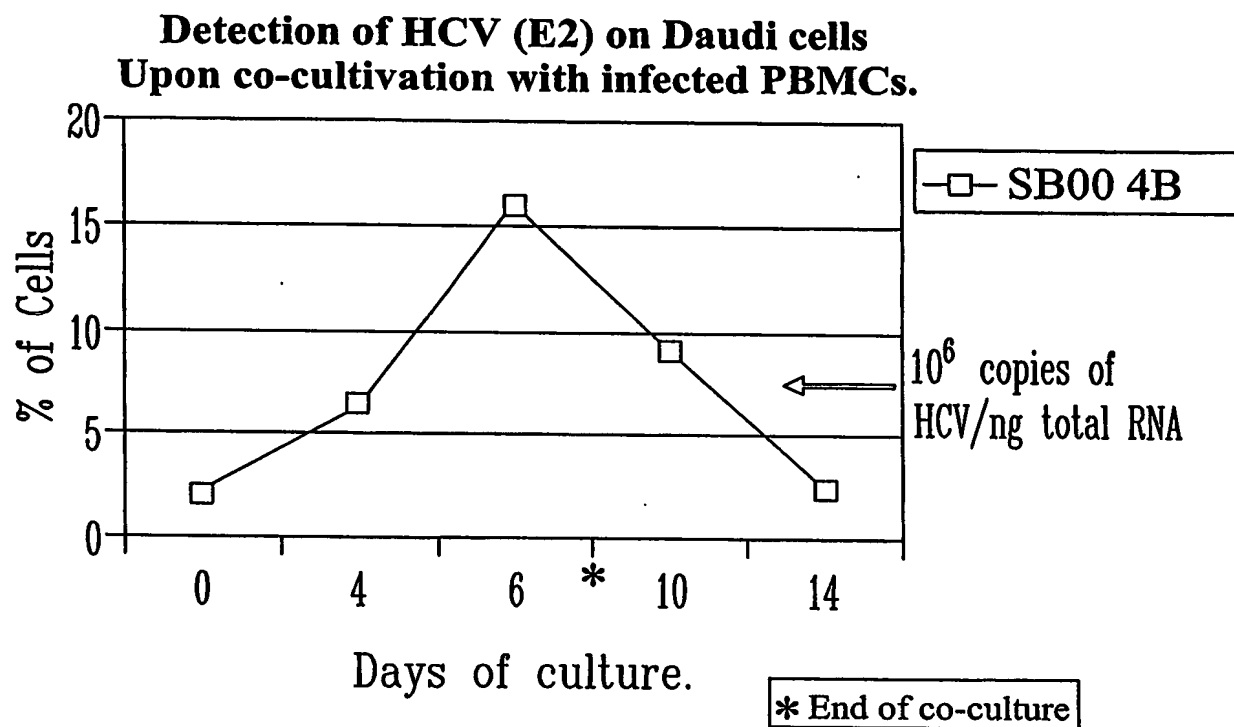
FIG. 18

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PHA Activation of PBMCs from patient SB004;
HCV is not in T cells

FIG. 19

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FIG. 20

Comparison of different activation treatments;

PBMCs from donor MLL-010

										T+B cells		Treatment	Days
										T cells (T1)	B cells (T2)		
										(T3)			
N	2	4	8	12	2	4	8	12	2	4	8		

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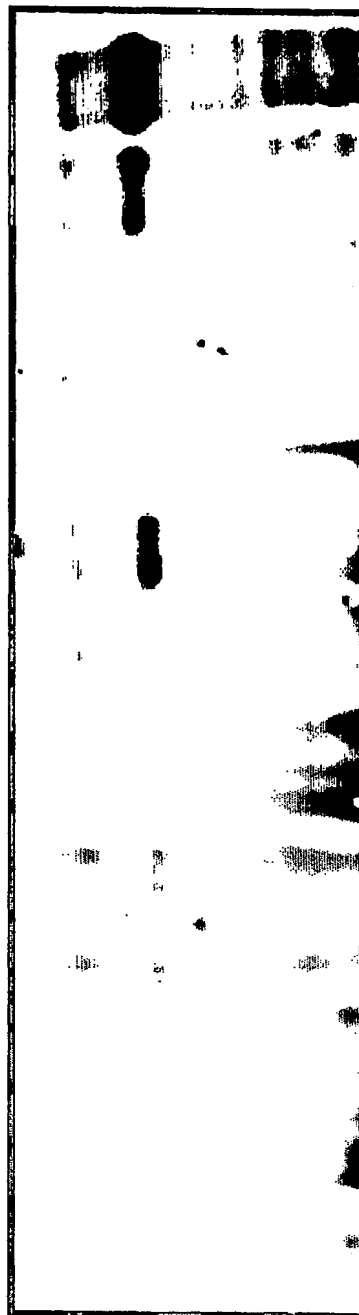


FIG. 21

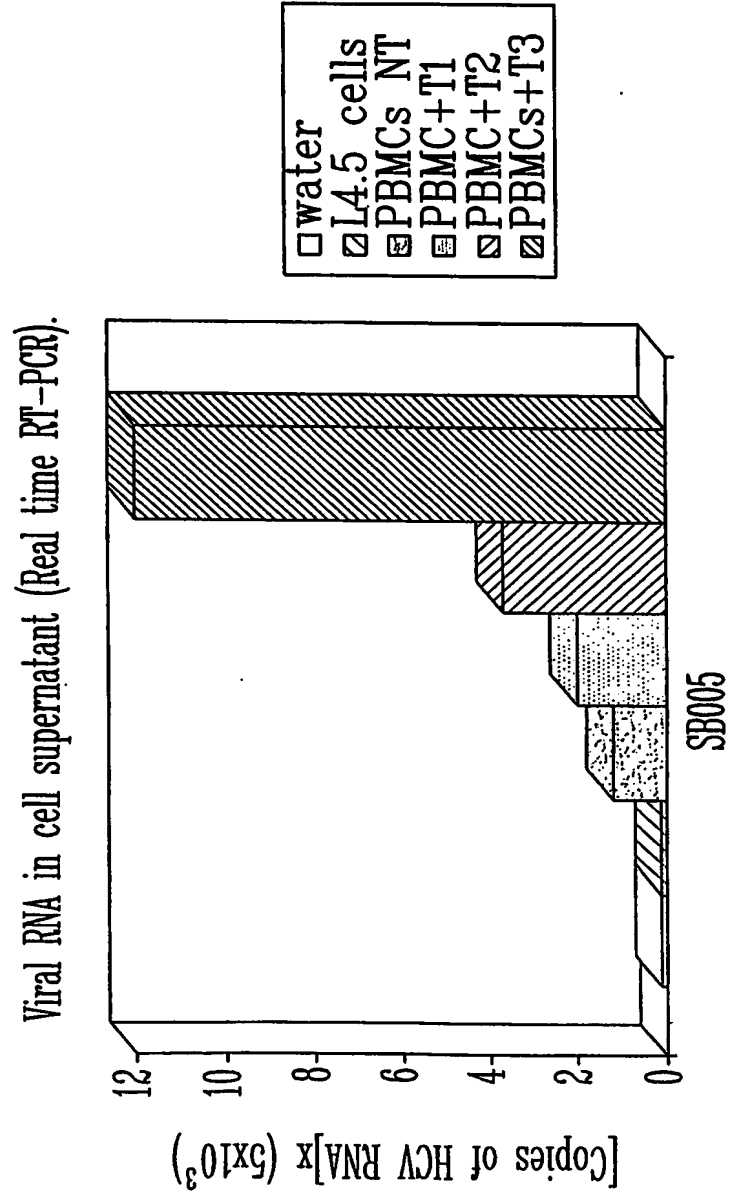


FIG. 22

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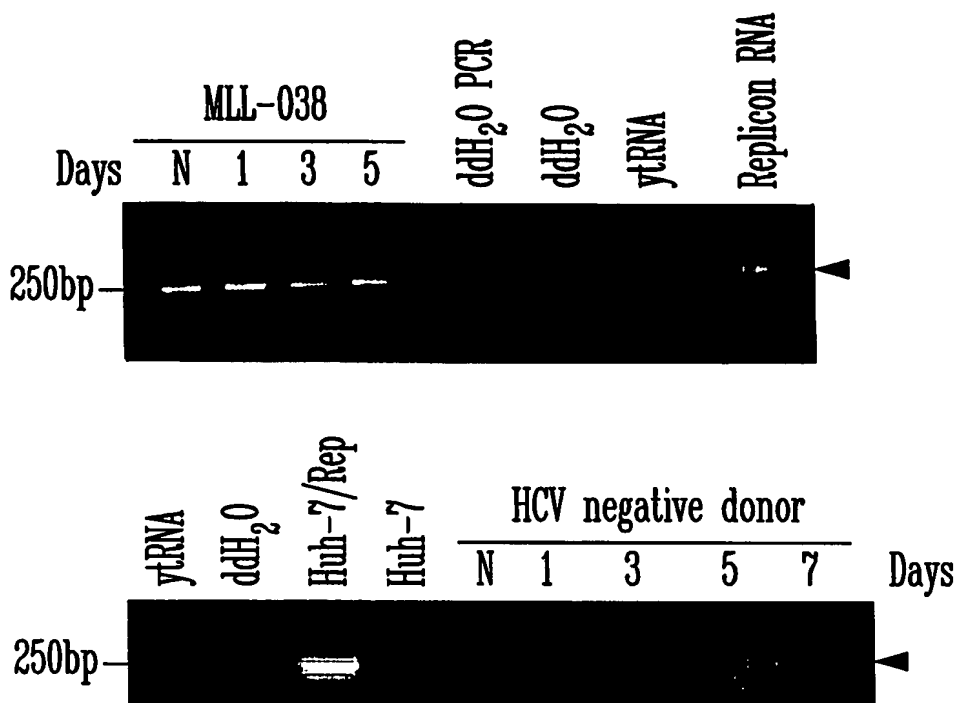


FIG. 23 A

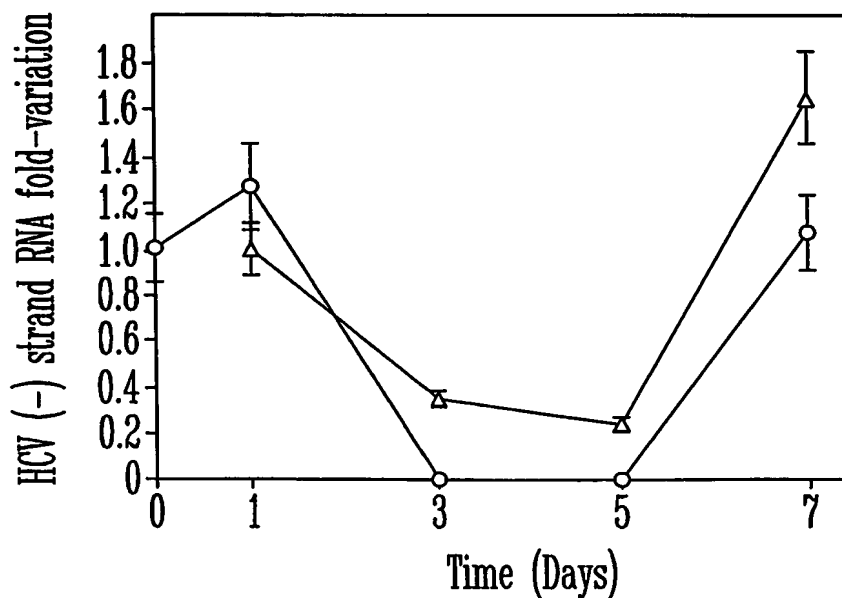


FIG. 23 B

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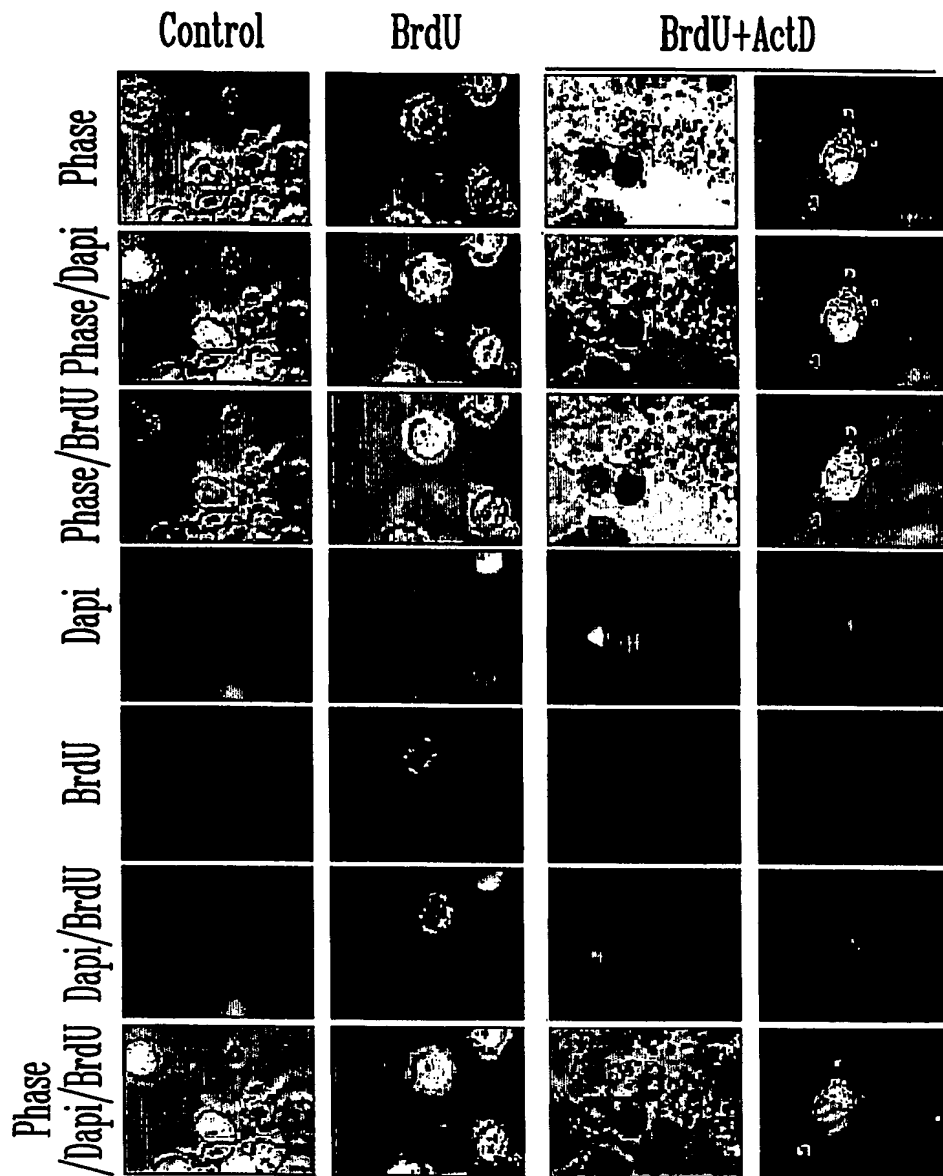


FIG. 23C

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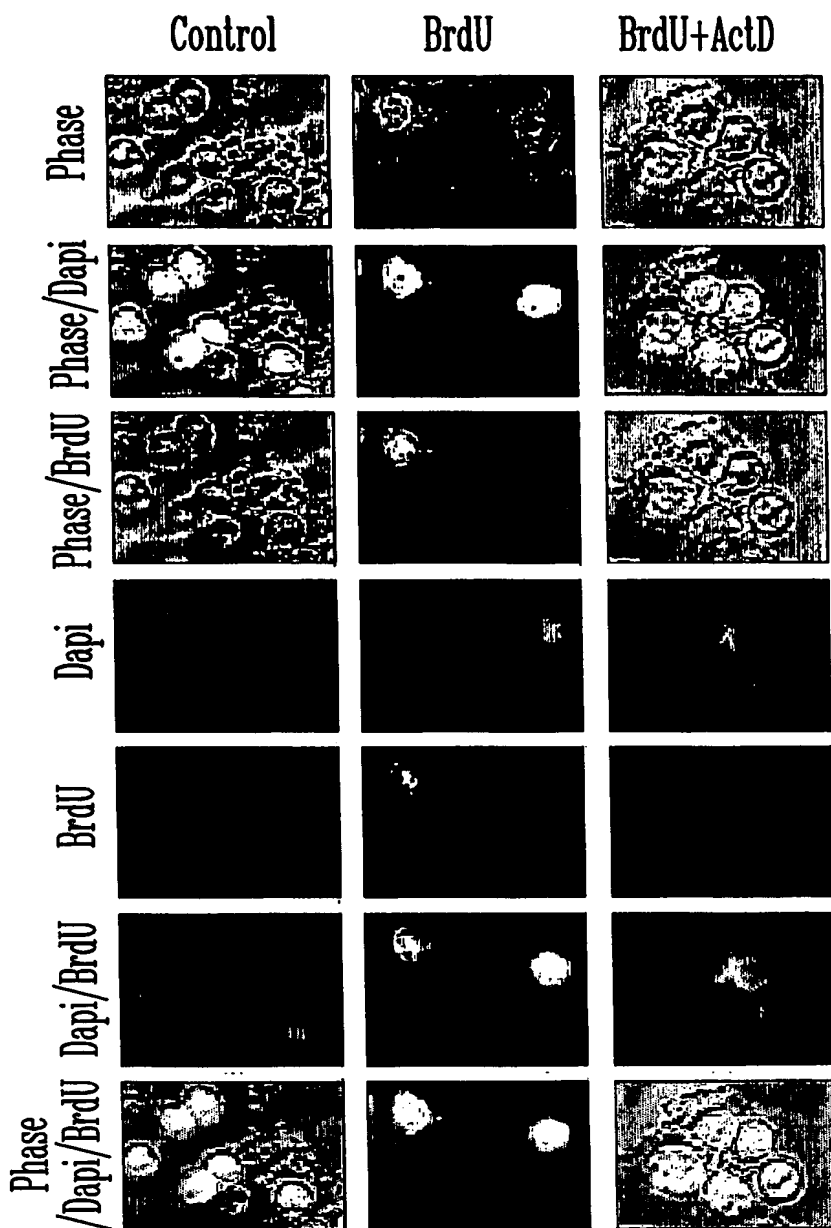
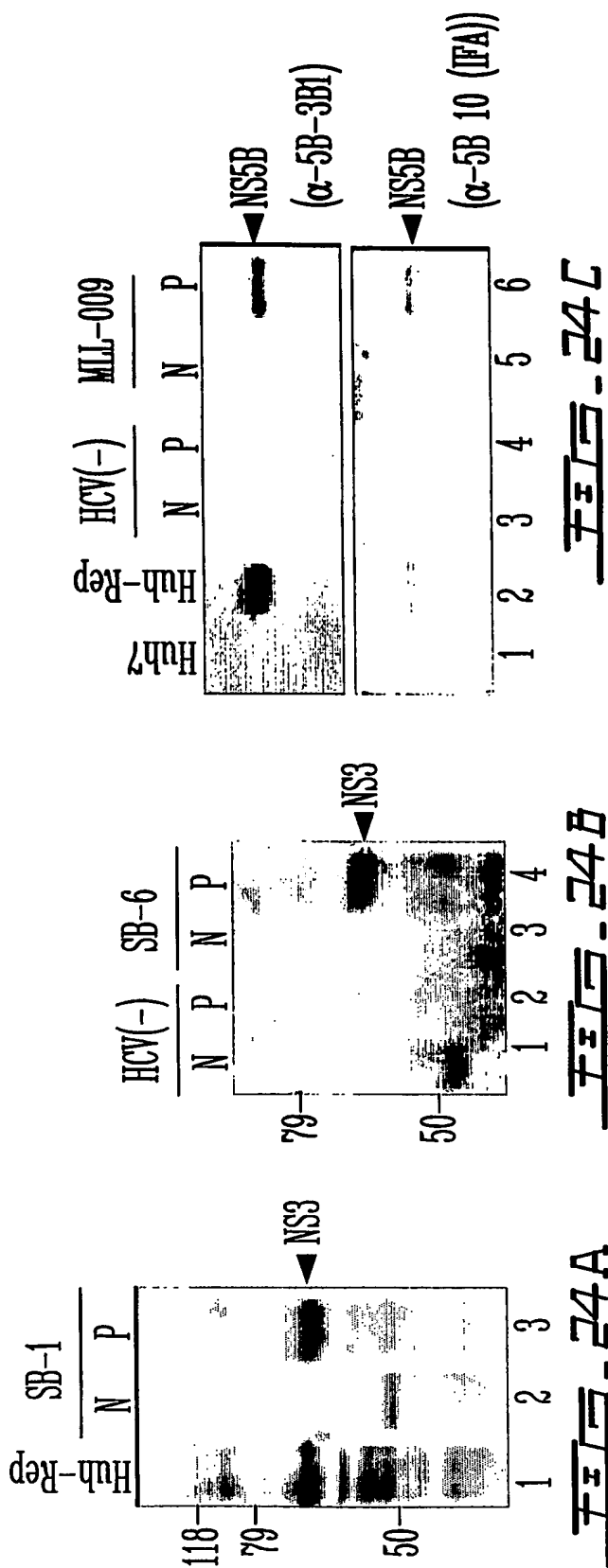
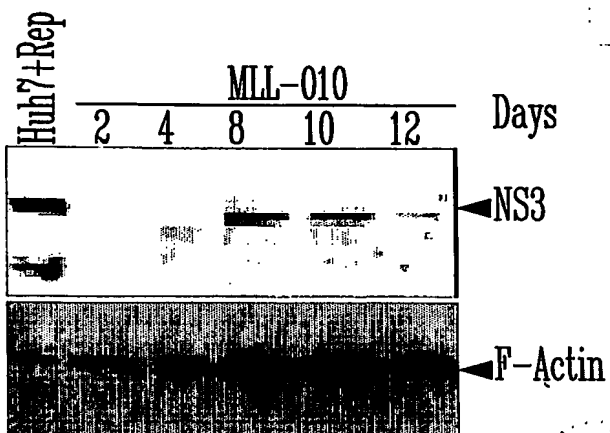
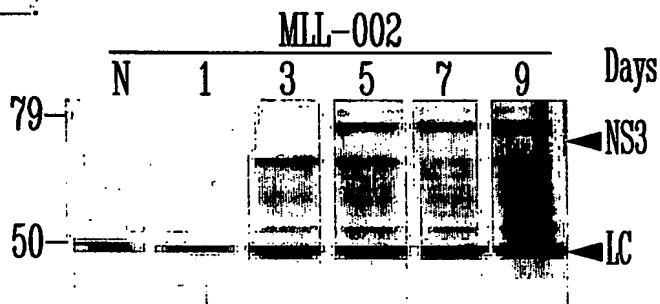
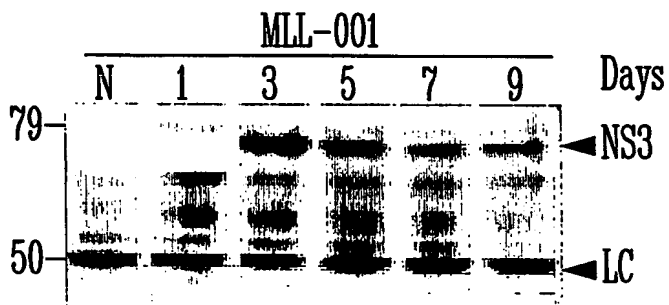
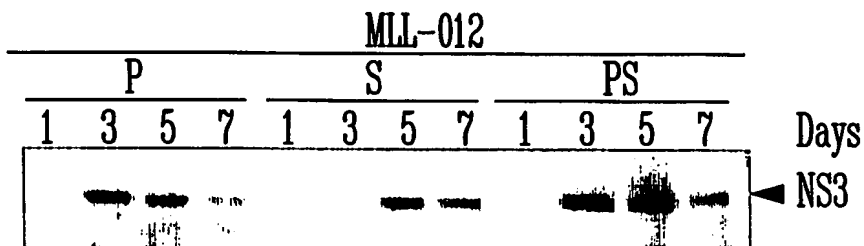
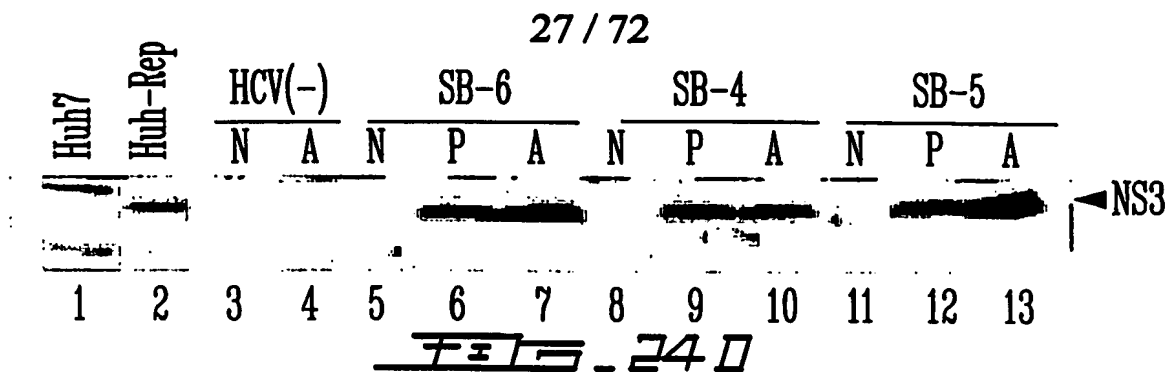


FIG. 23D





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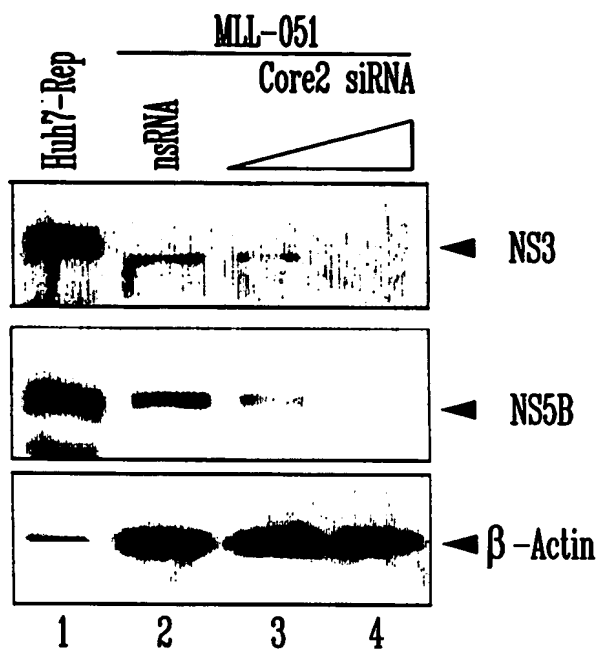


FIG. 24I

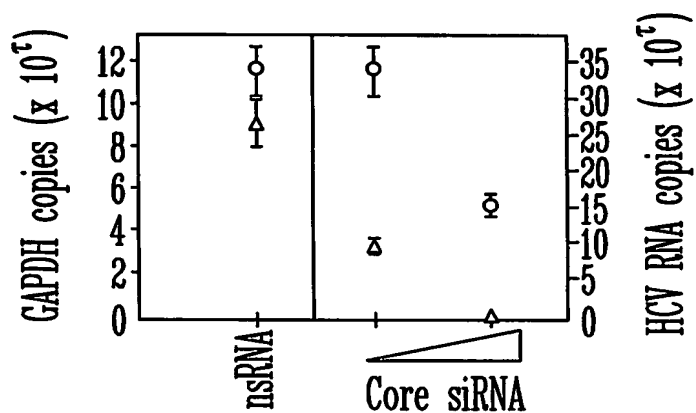


FIG. 24J

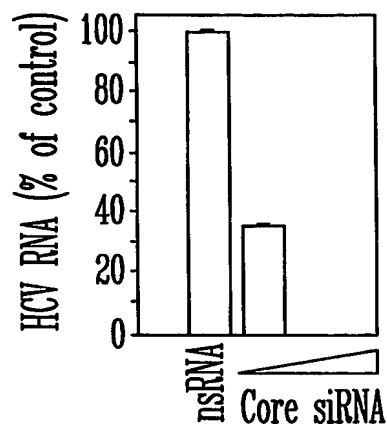


FIG. 24K

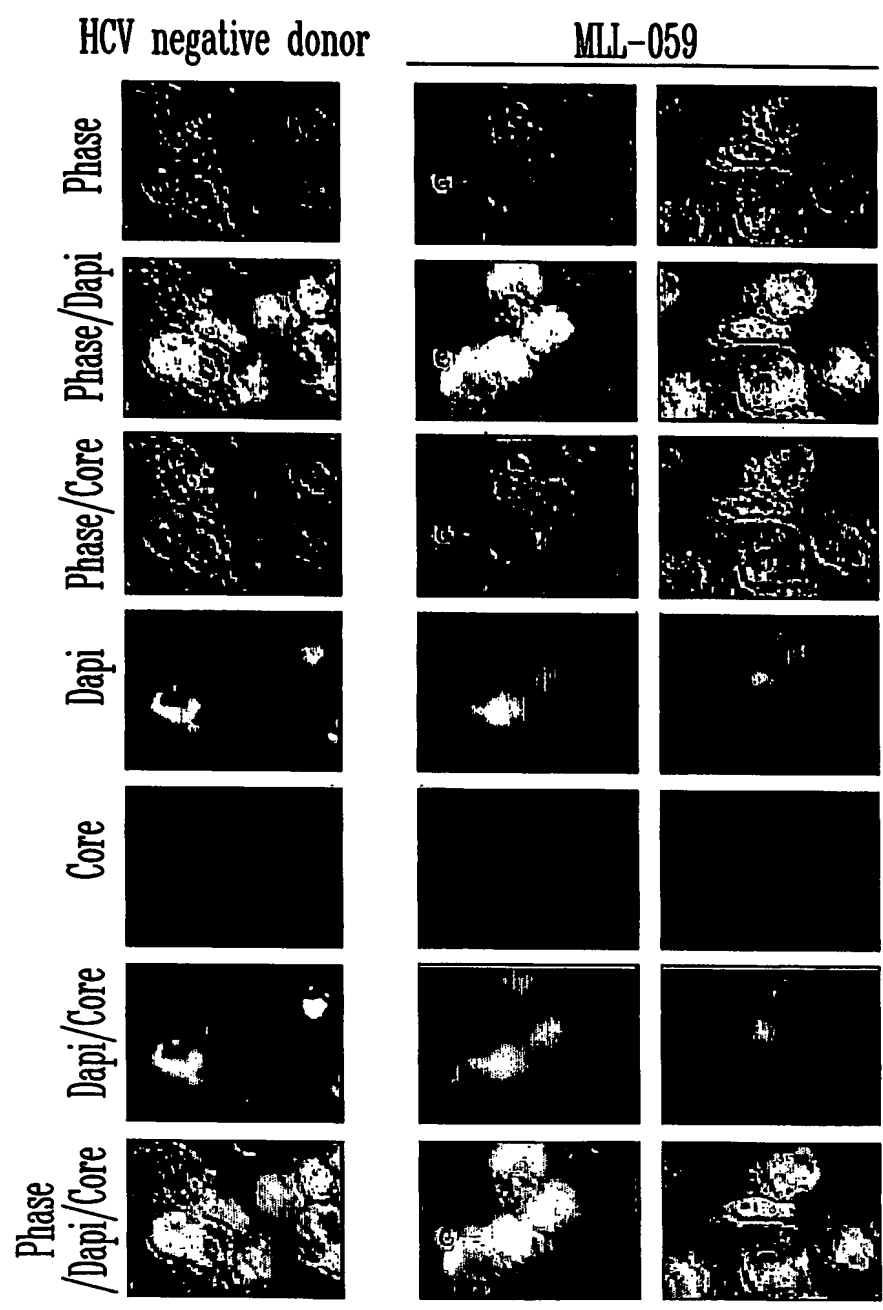


FIG. 25

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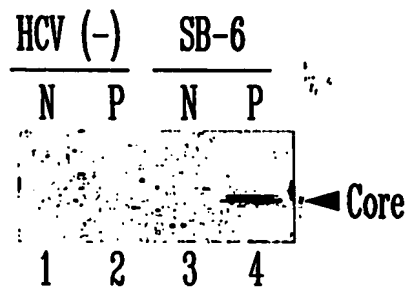
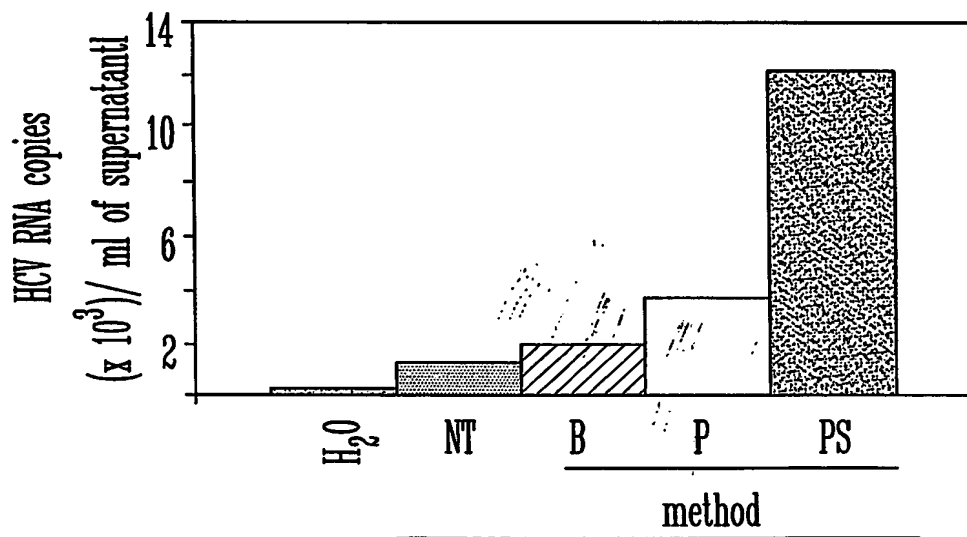


FIG. 26A



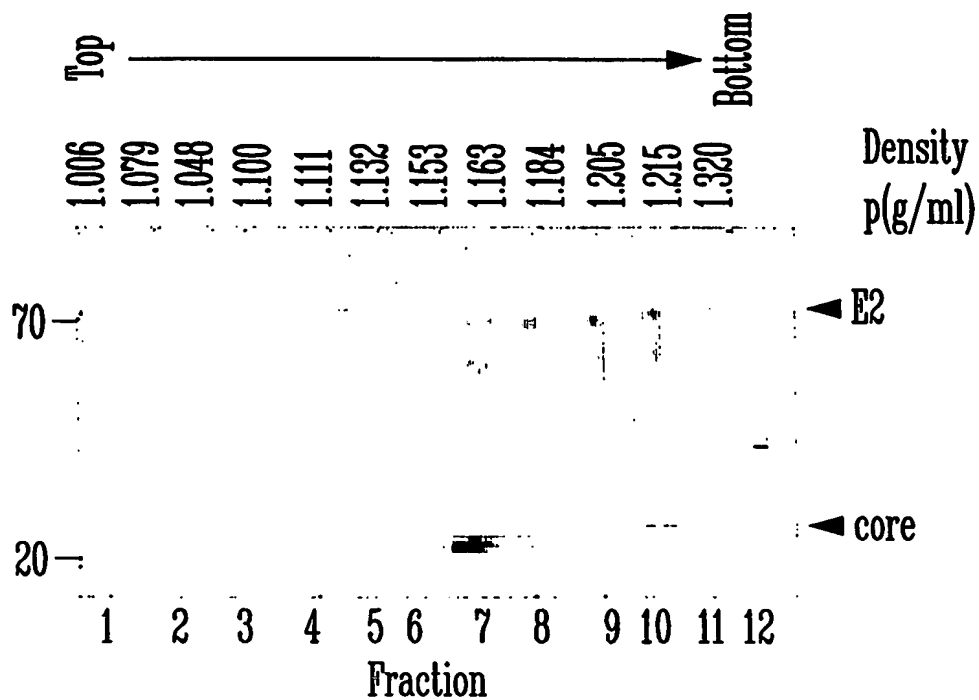
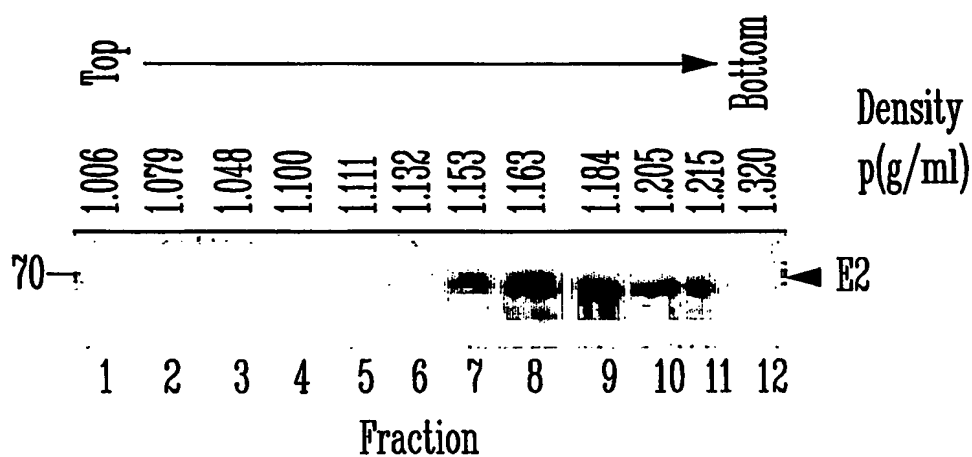
FIG. 26B



SB-5

FIG. 26C

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FIG. 26DFIG. 26E

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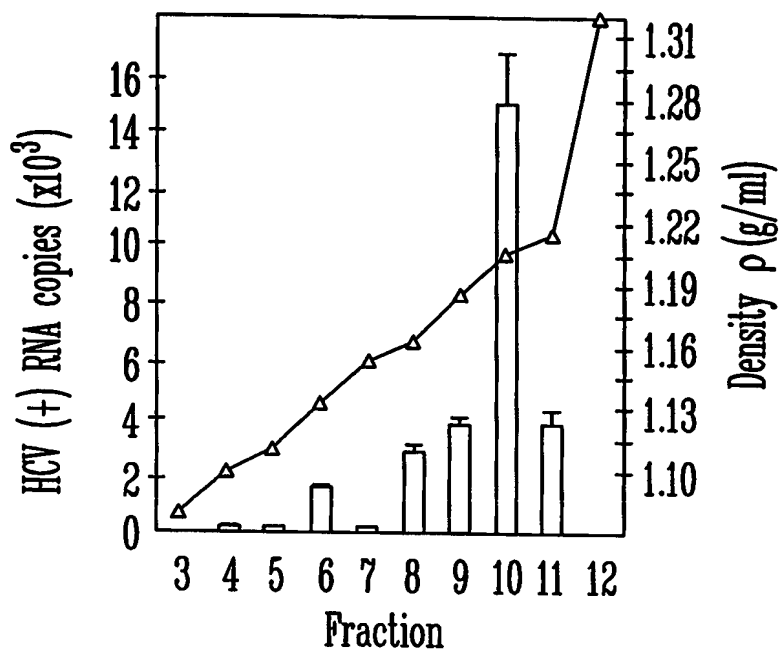


FIG. 26F

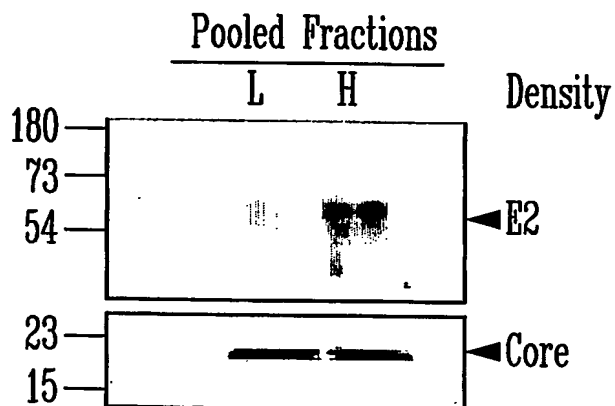


FIG. 26G

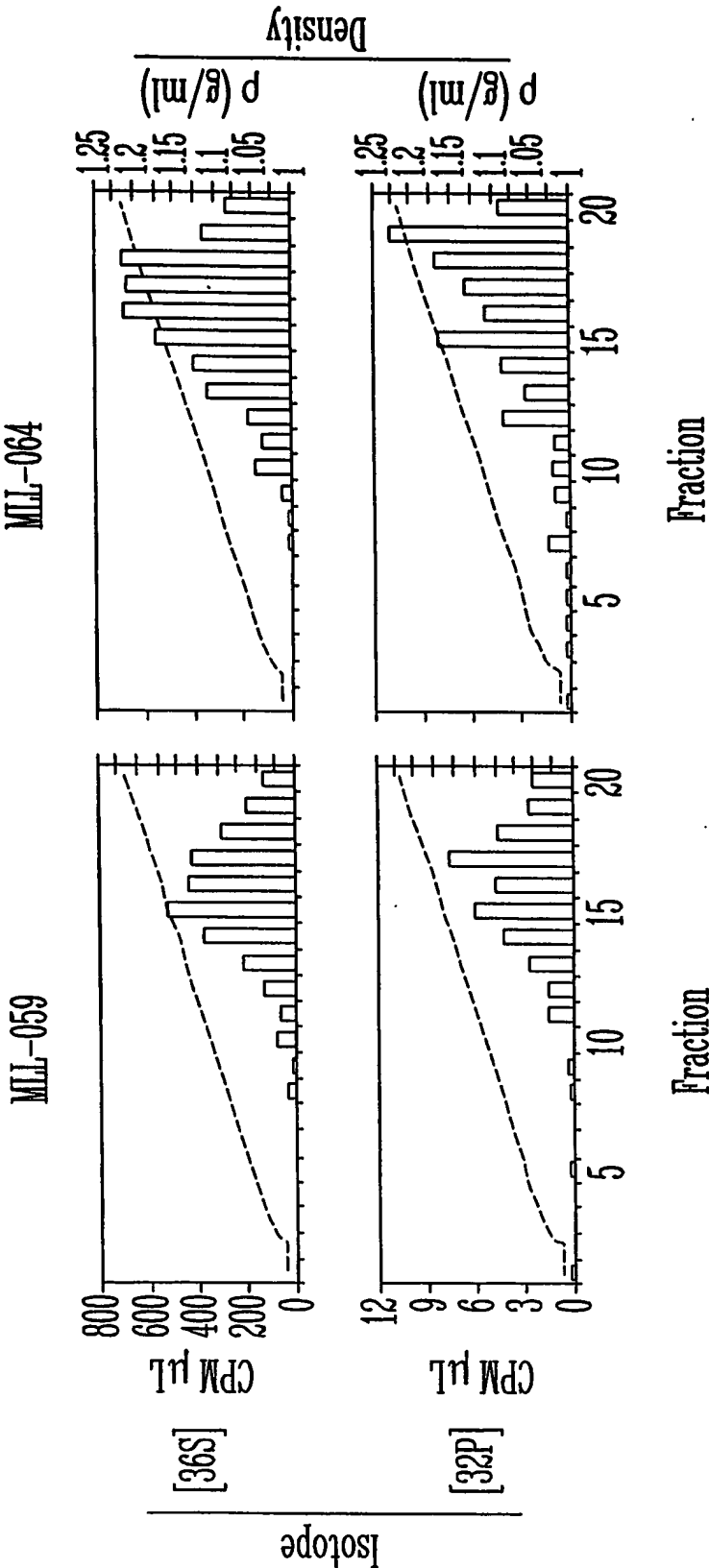


FIG. 25H

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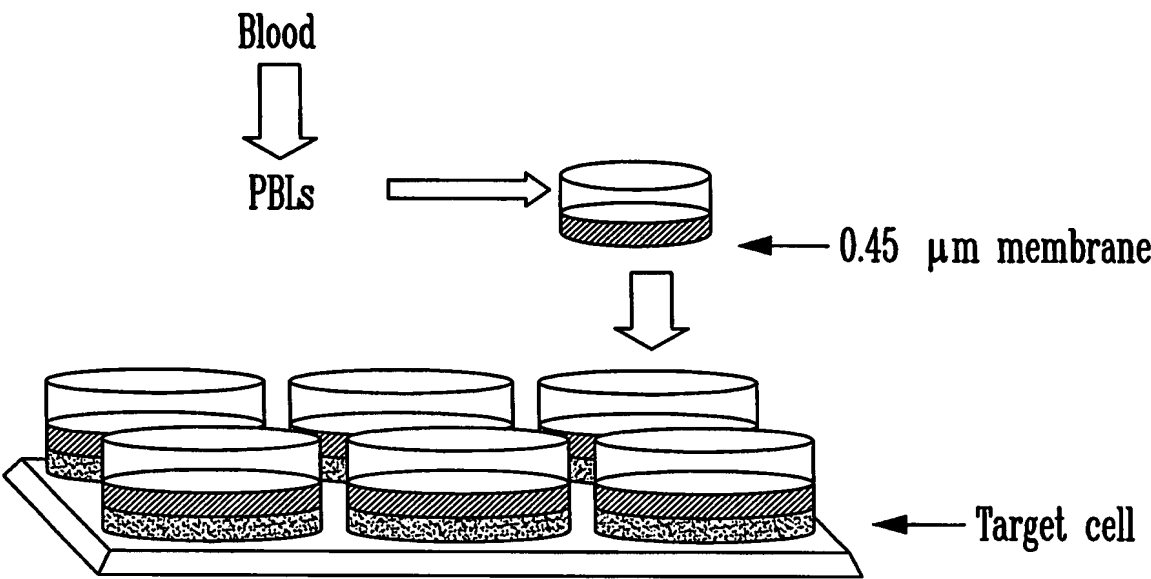


FIG. 27A

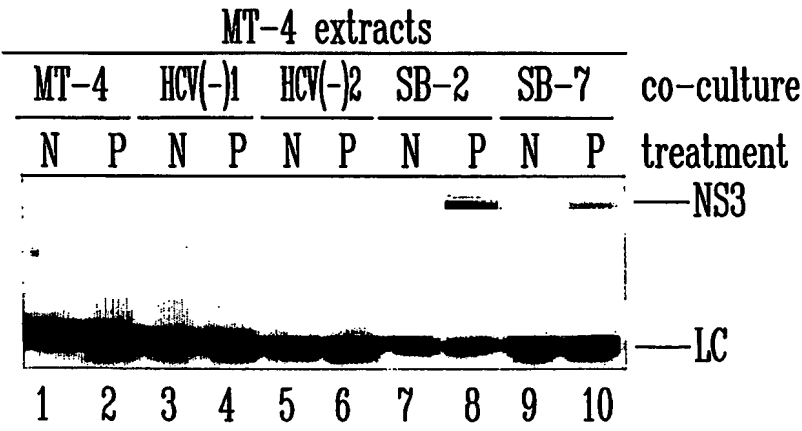
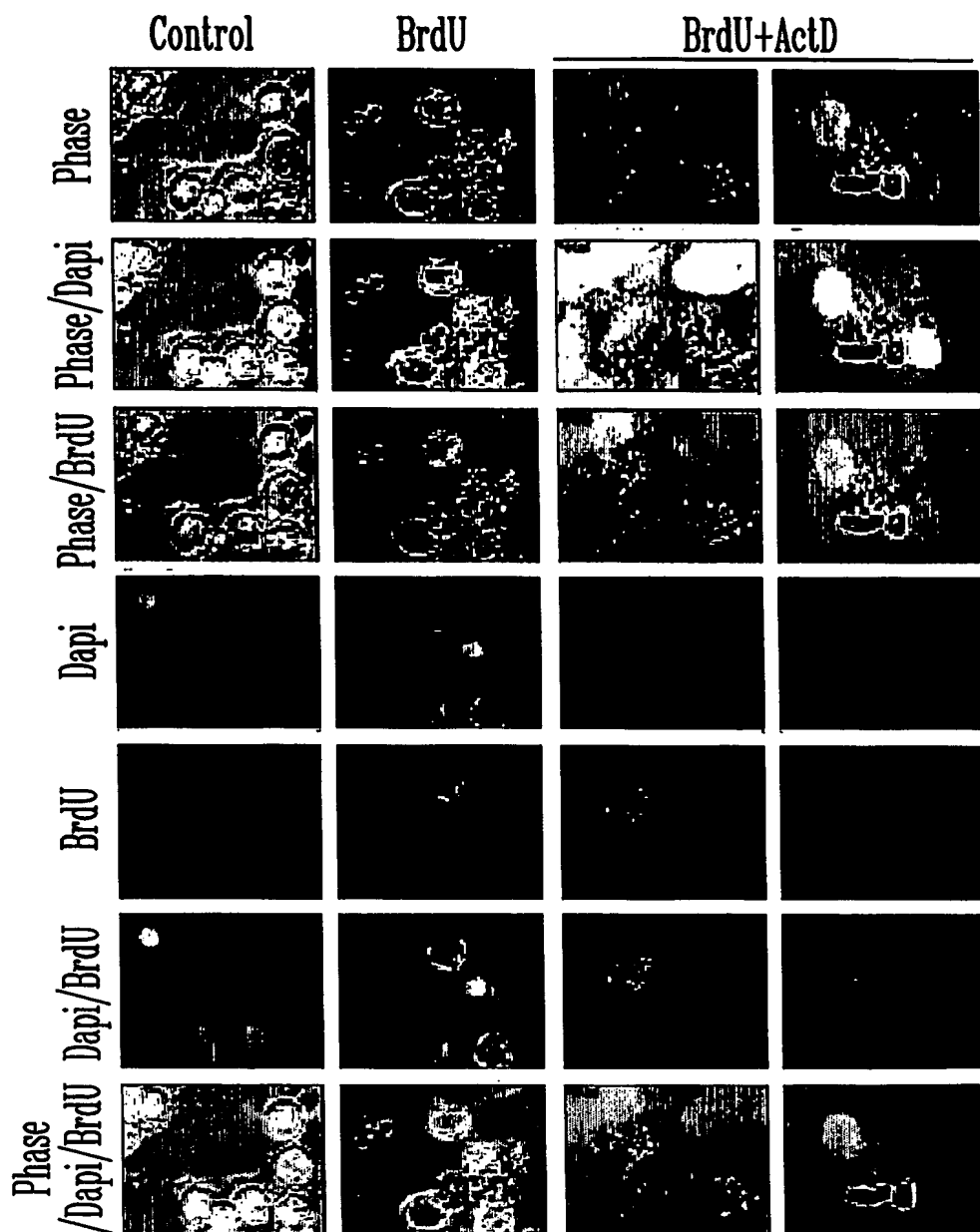


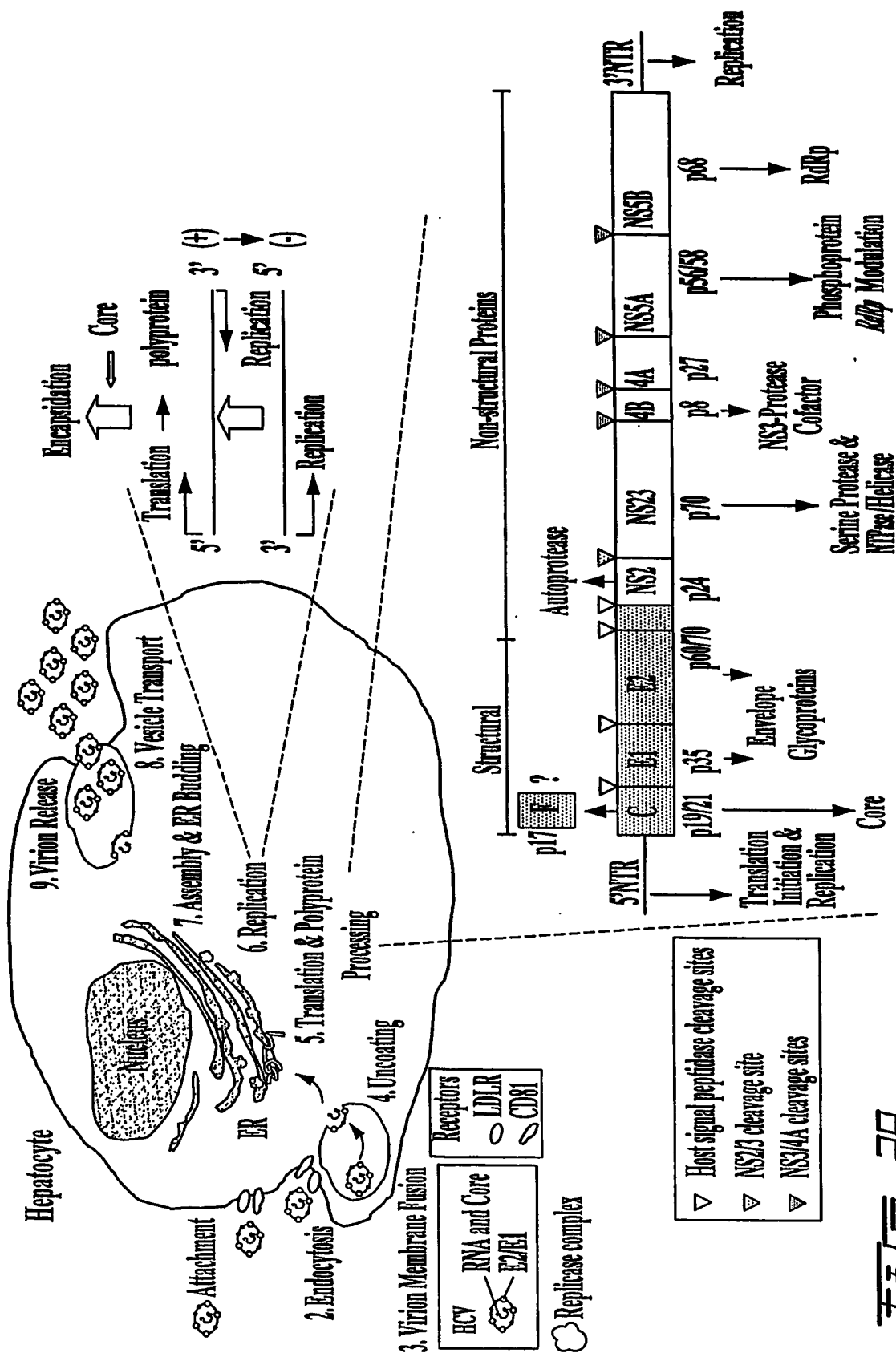
FIG. 27B

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HCV Replication Cycle



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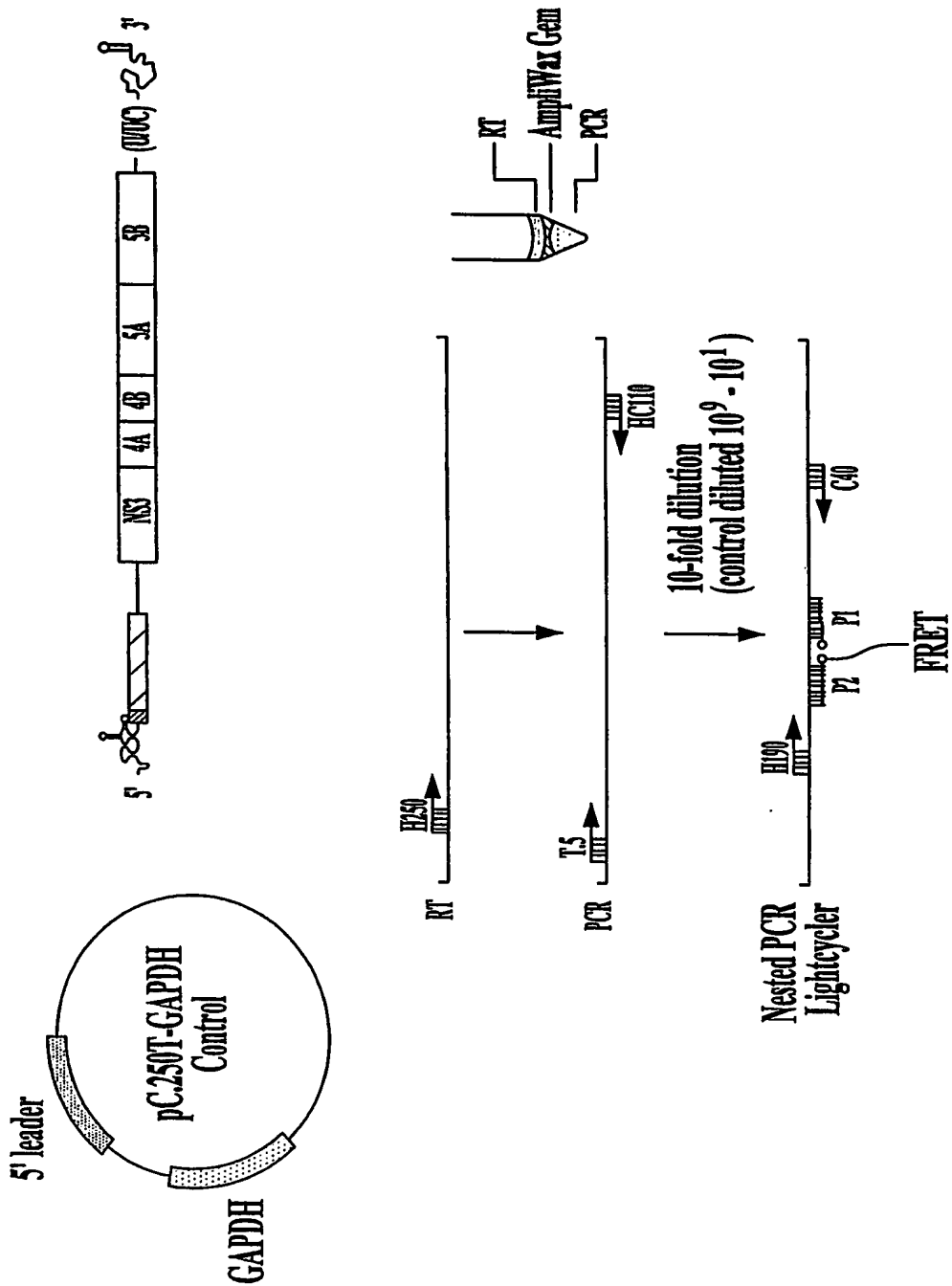


FIG. 30A

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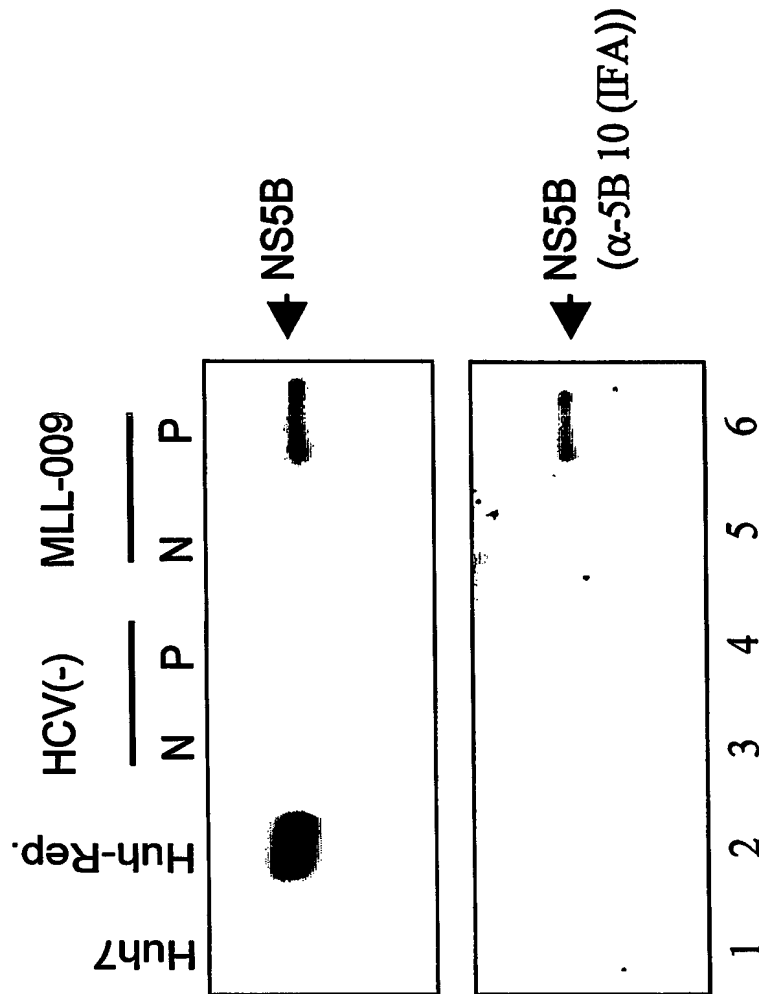


Fig. 30B

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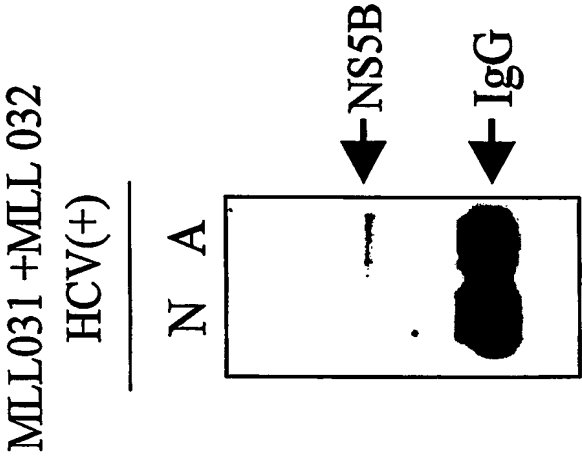
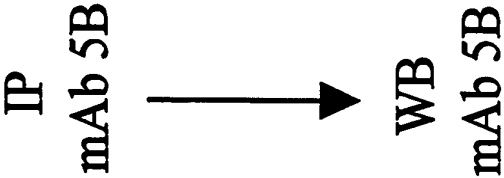


FIG. 31

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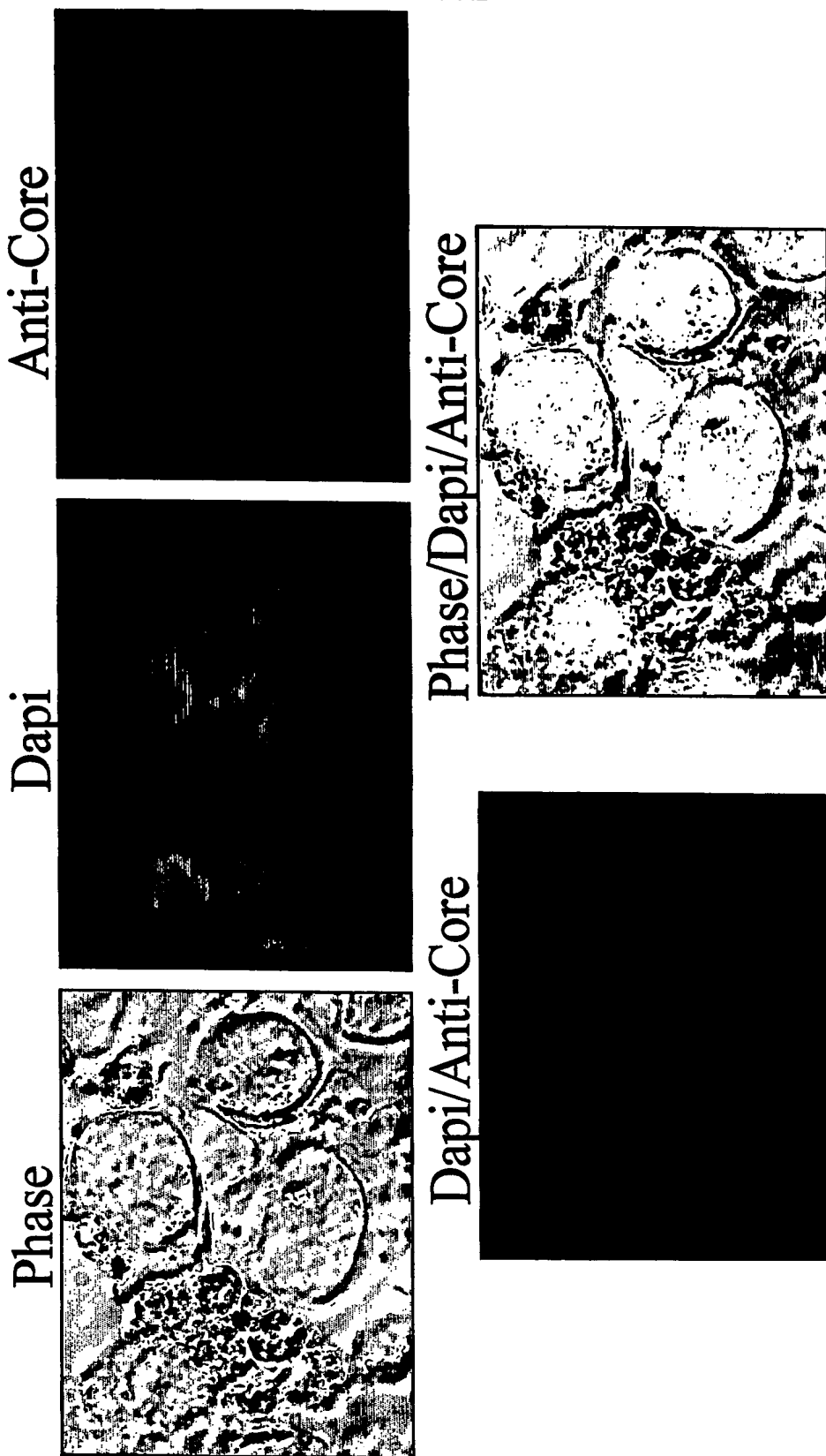


FIG. 32

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FIG. 33

Anti-Core



Dapi



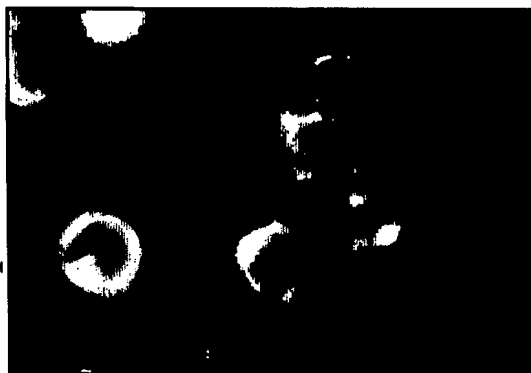
Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core



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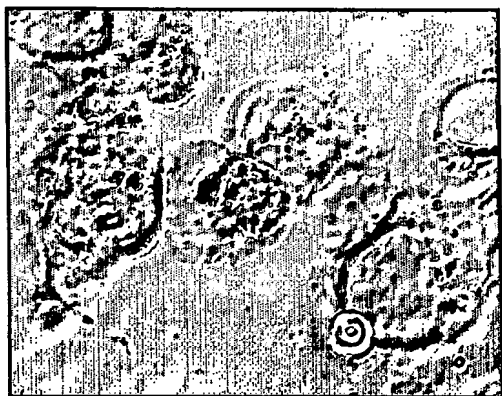
Anti-Core



Dapi



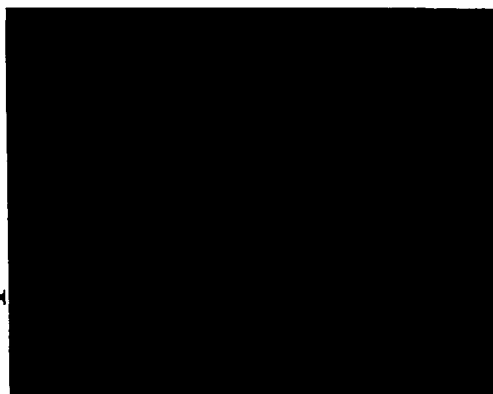
Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core



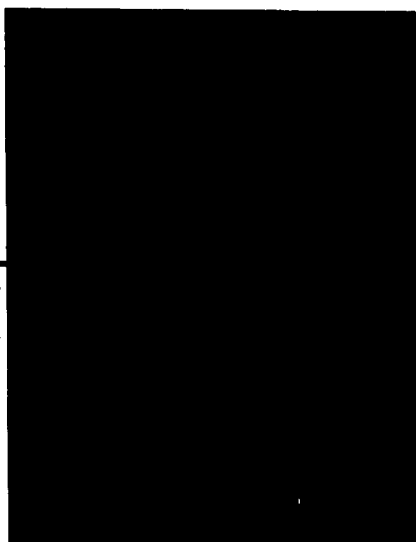
~~FIG. 34~~

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Anti-Core



Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core

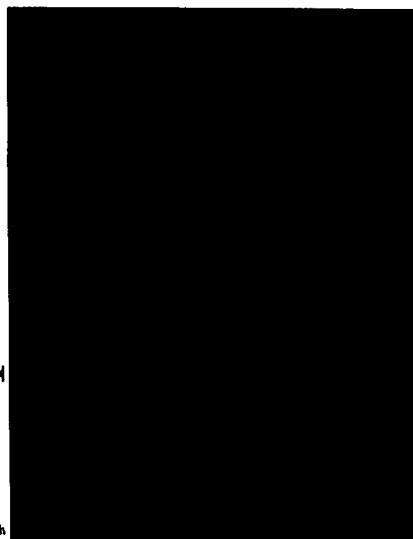


FIG. 35

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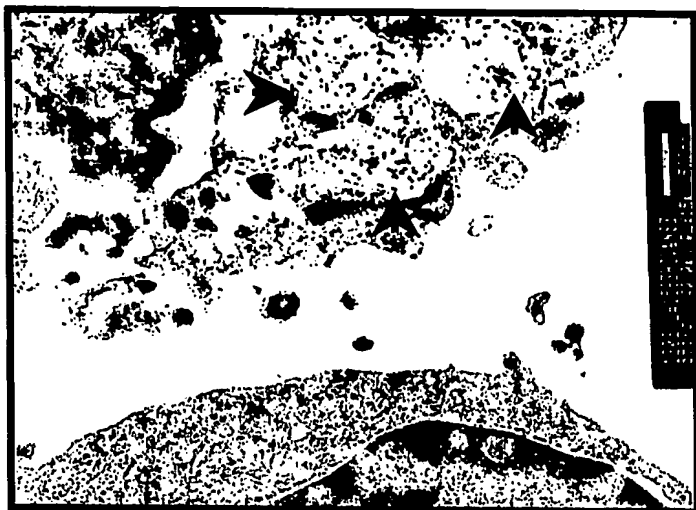


FIG. 36C



FIG. 36B



FIG. 36A

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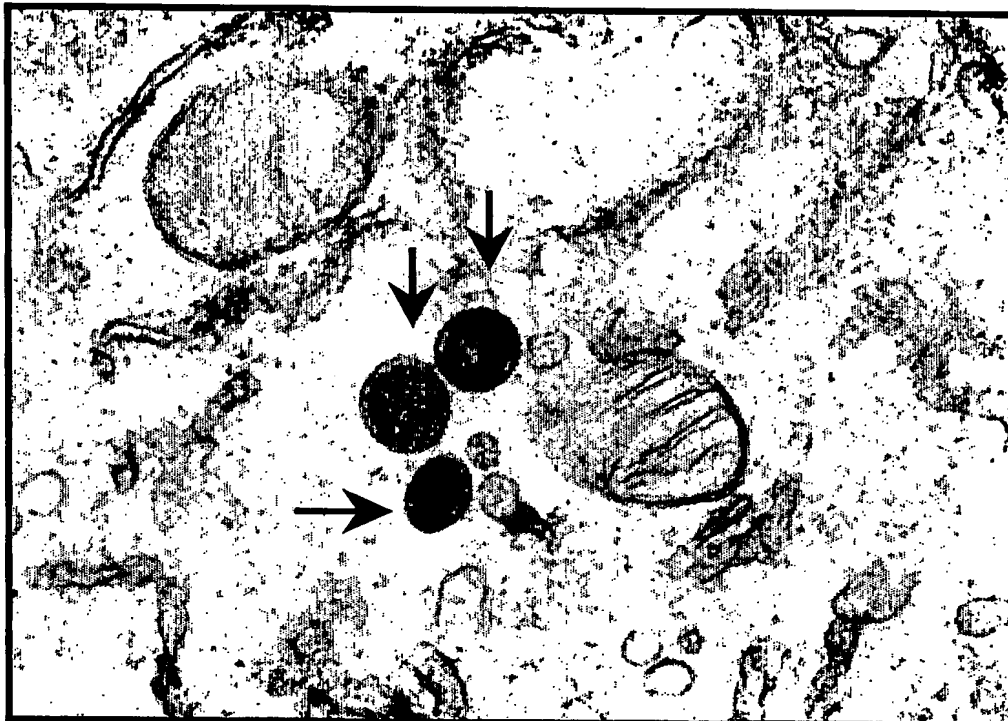
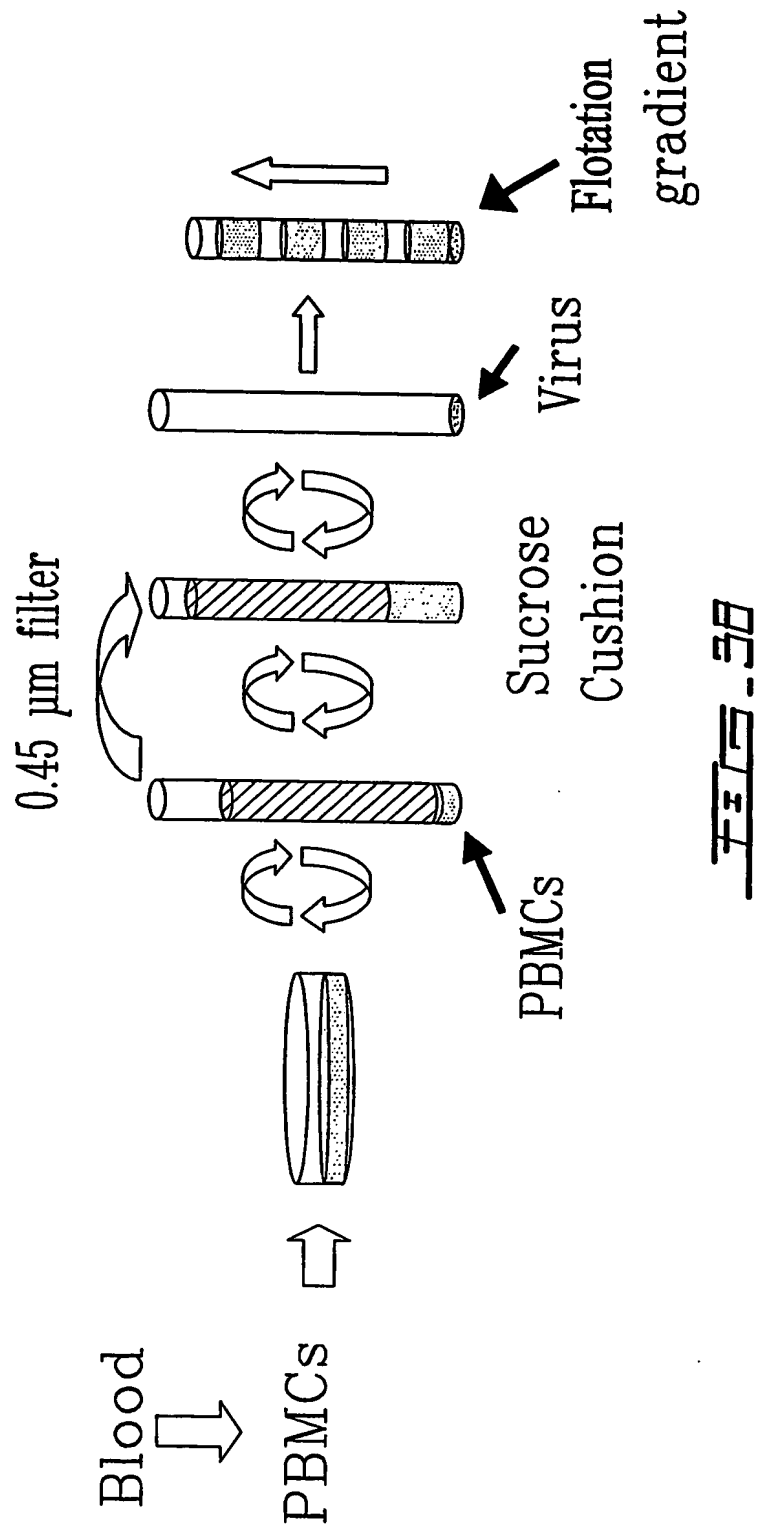


FIG. 37

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Virus partial purification.

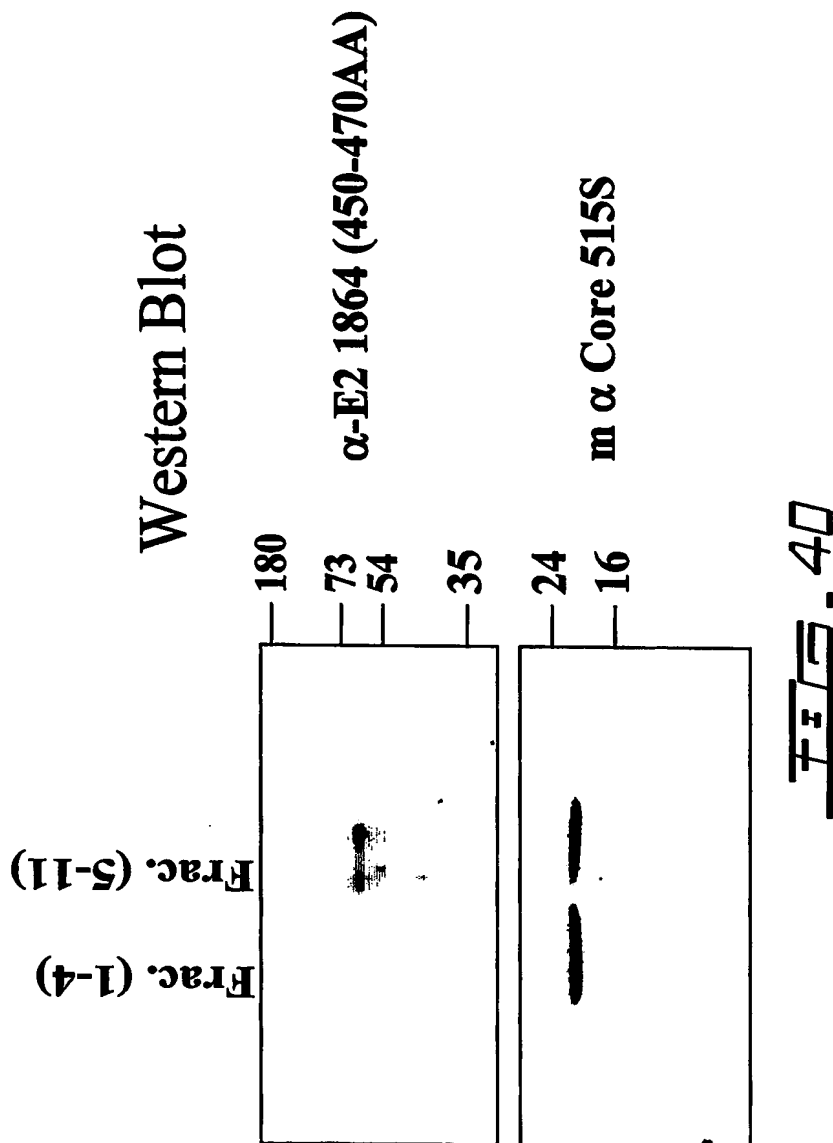


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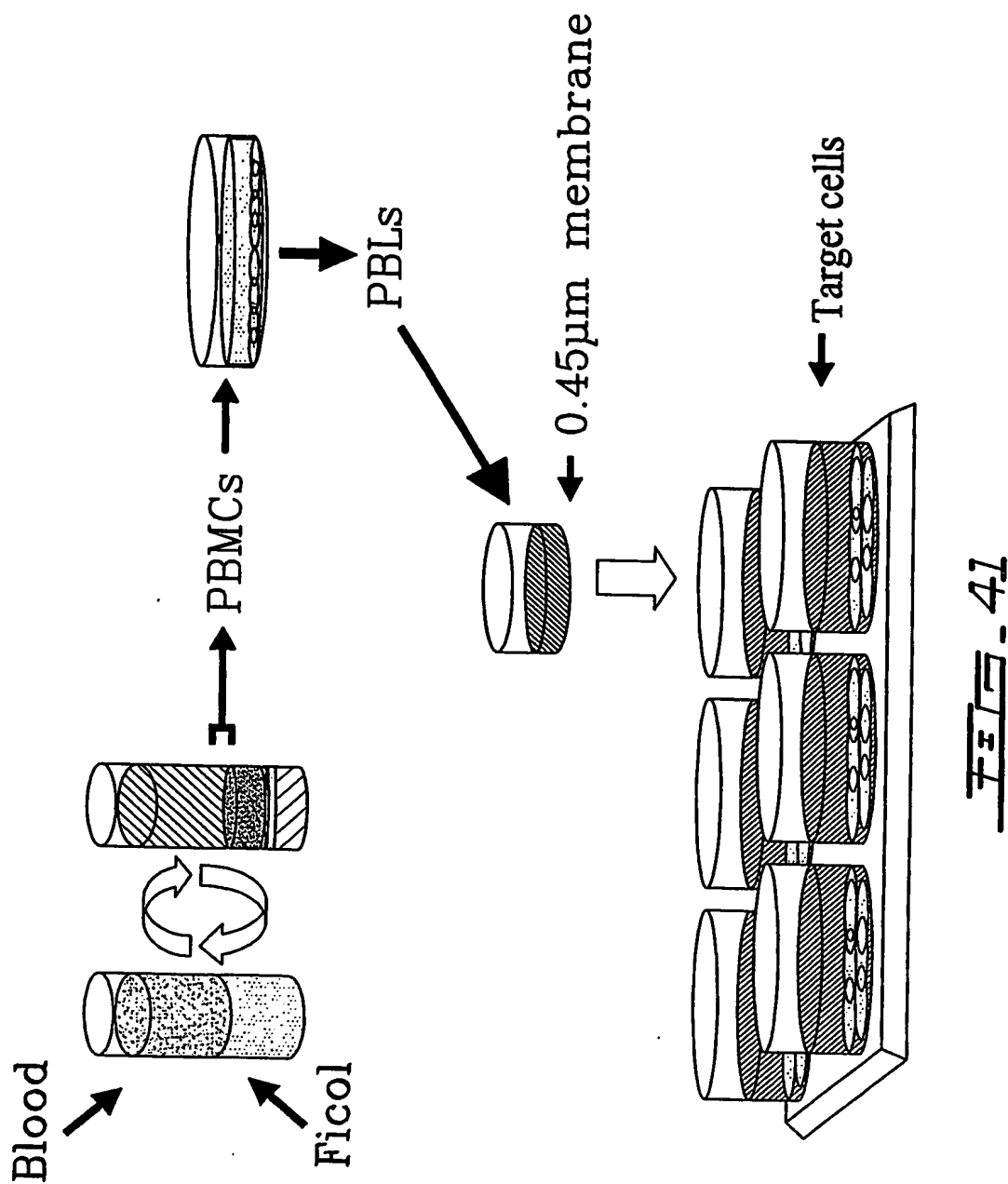
Density Range (g/ml)	Source	Reference
1.15-1.20	HCV-LP in VSV vector	J.Virol (2002) 76, 12325.
1.14-1.18	HCV-LP in insect cells	J. Virol (1998) 72, 3827.
1.12-1.17	Plasma chimps	J. Gen.Virol (1994) 75, 1755
1.09-1.21	Plasma chimps	J.Med.Virol (1991), 34, 206.
1.13-1.17	Plasma chimps	J.Virol (1993) 67, 1953
1.063-1.21	Serum infected donors	J Med Virol (2002) 68, 335
1.11-1.215	HCV(+) PBMCs	-----

FIG. 39

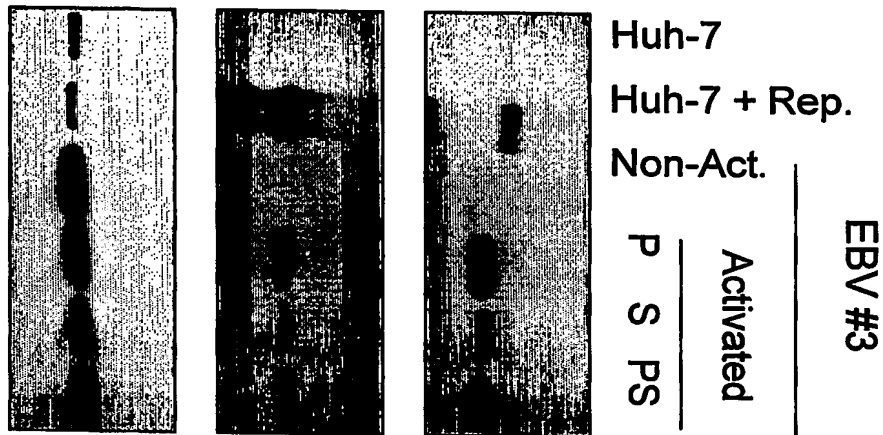
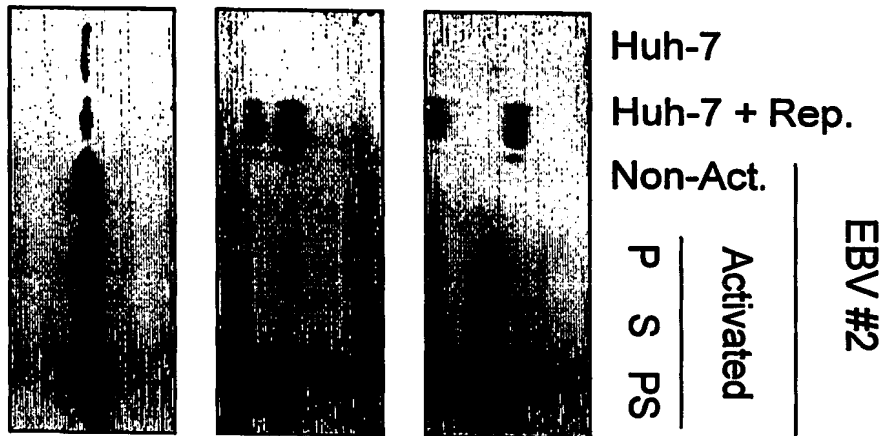
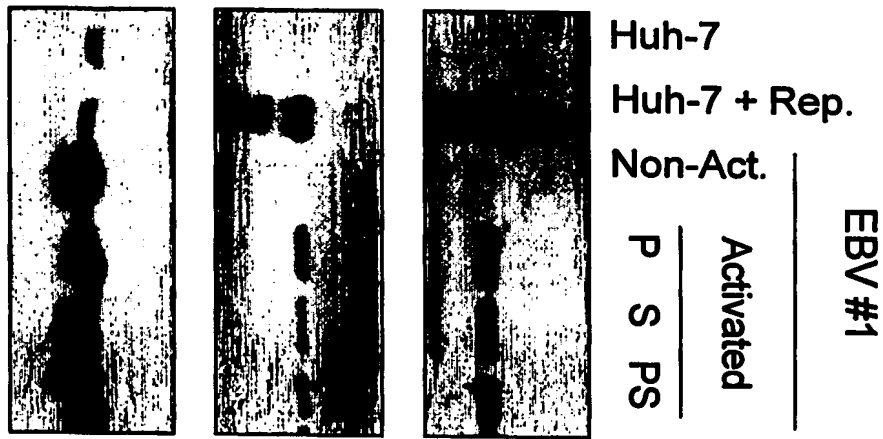
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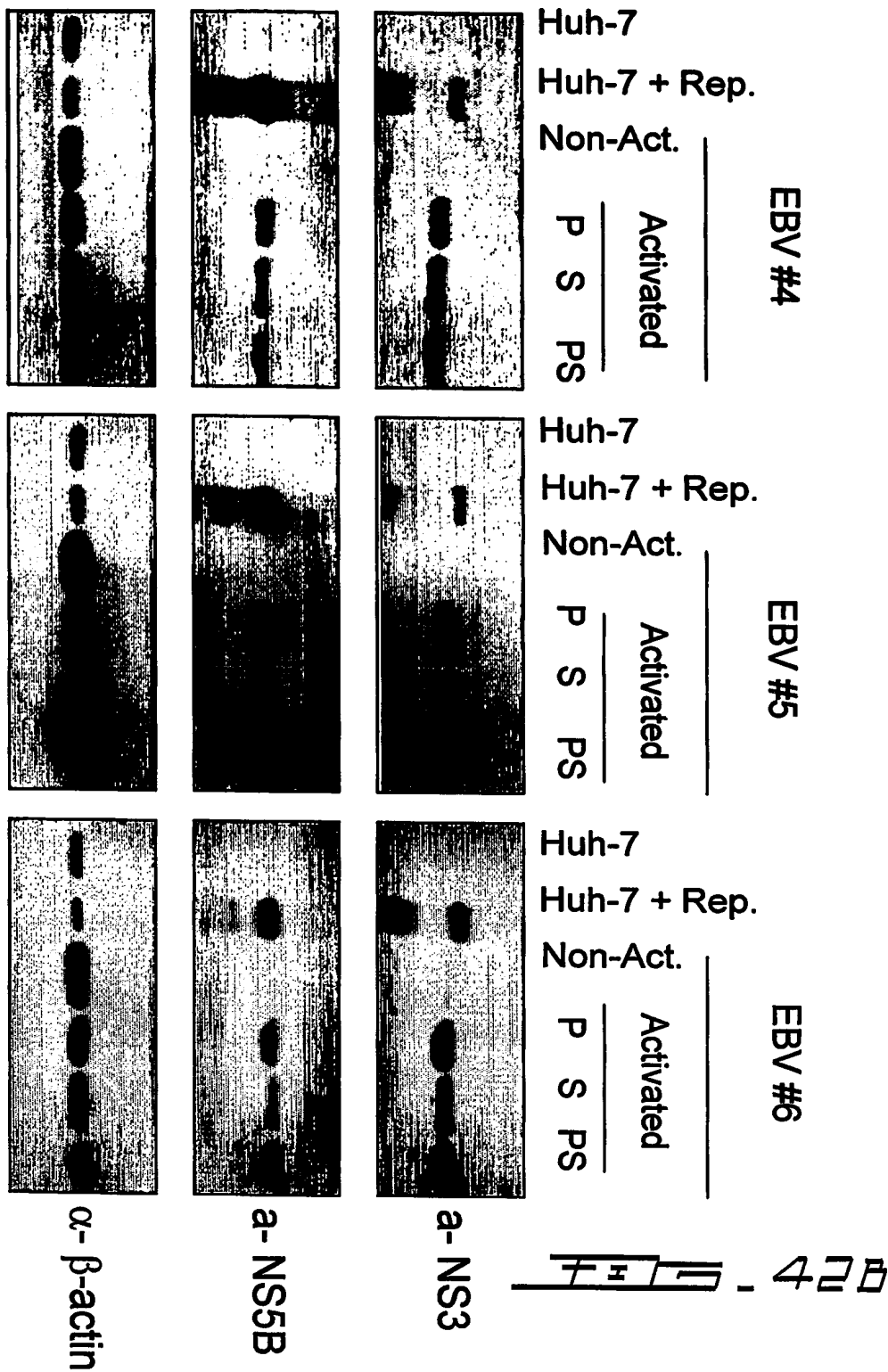
α - β -actin

α -NS5B

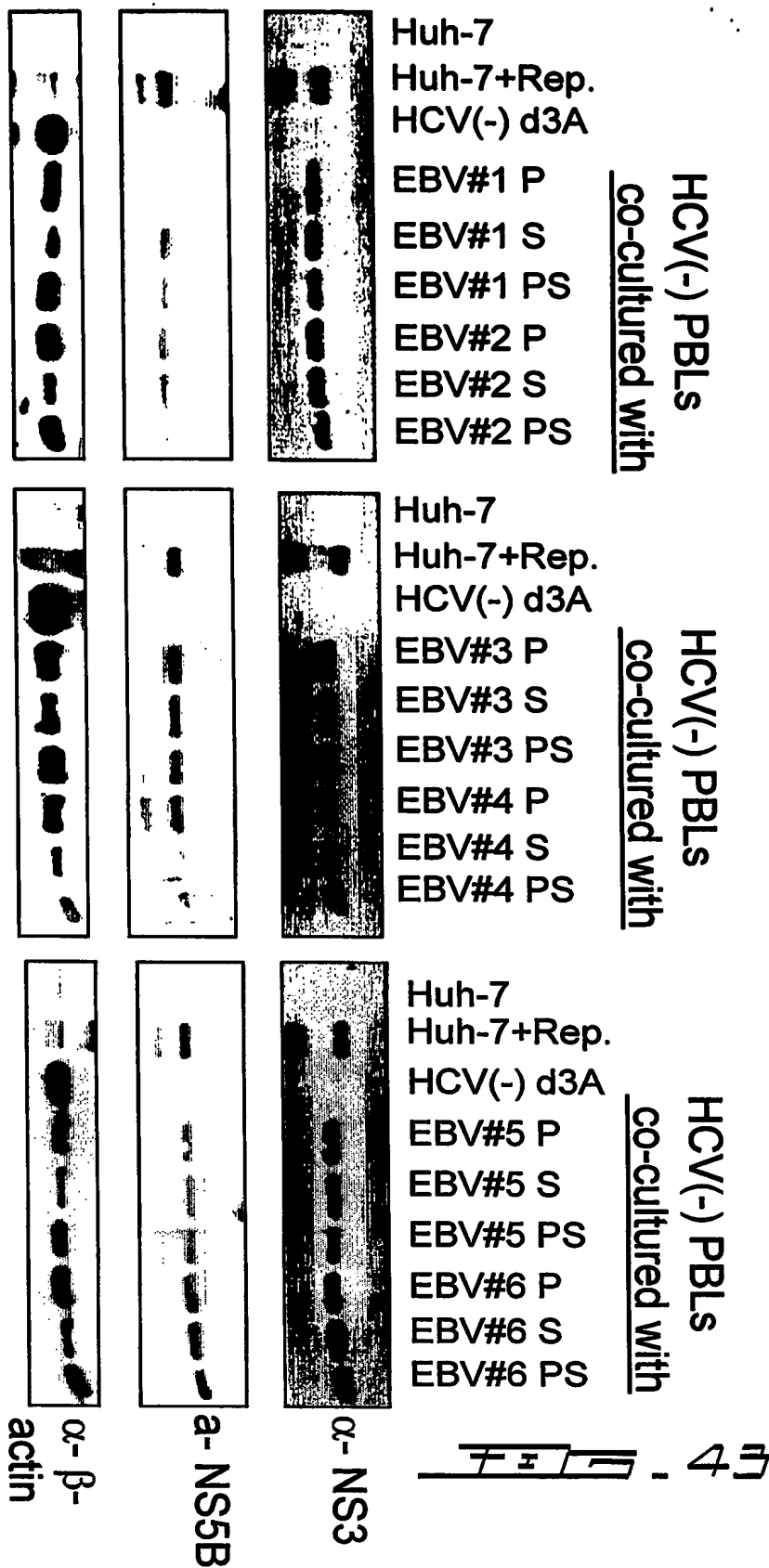
α -NS3

Fig. 42A

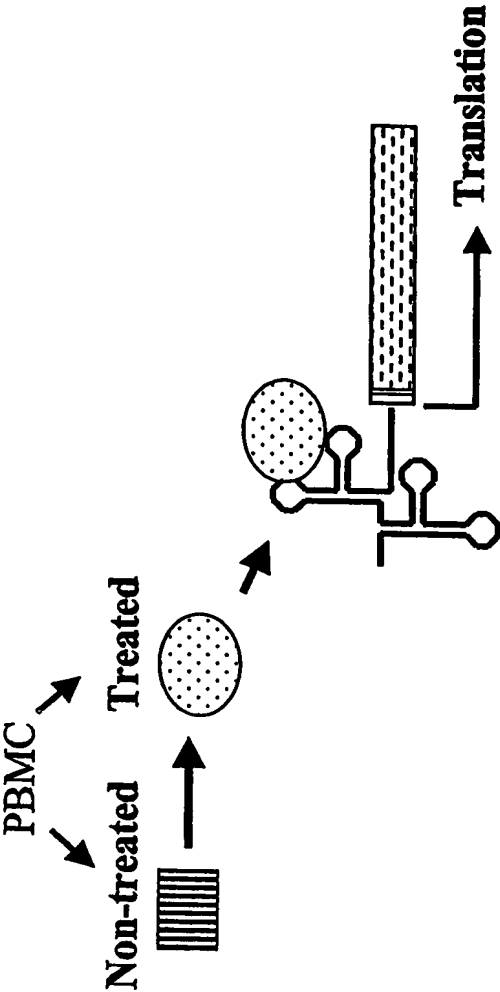
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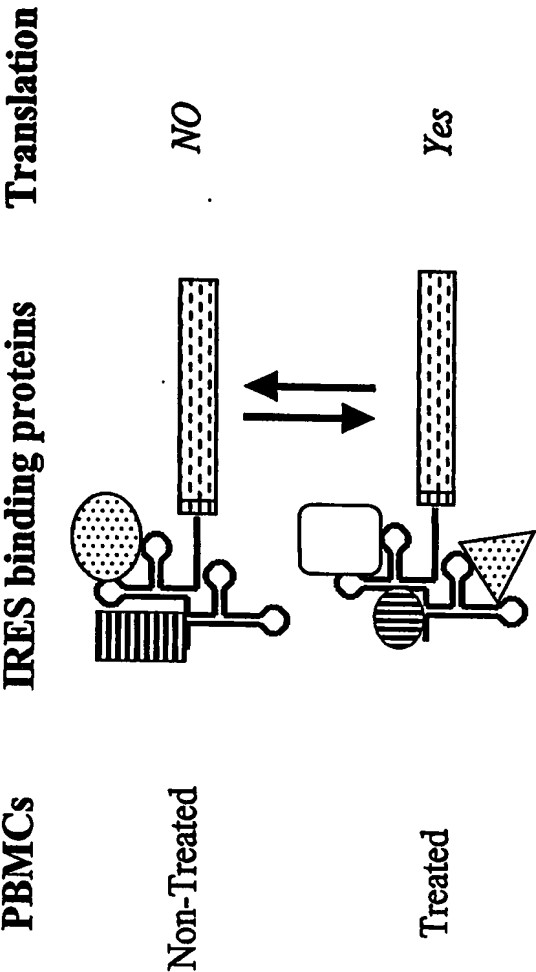
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I- Translation Activator.



II- Translation inhibitor.



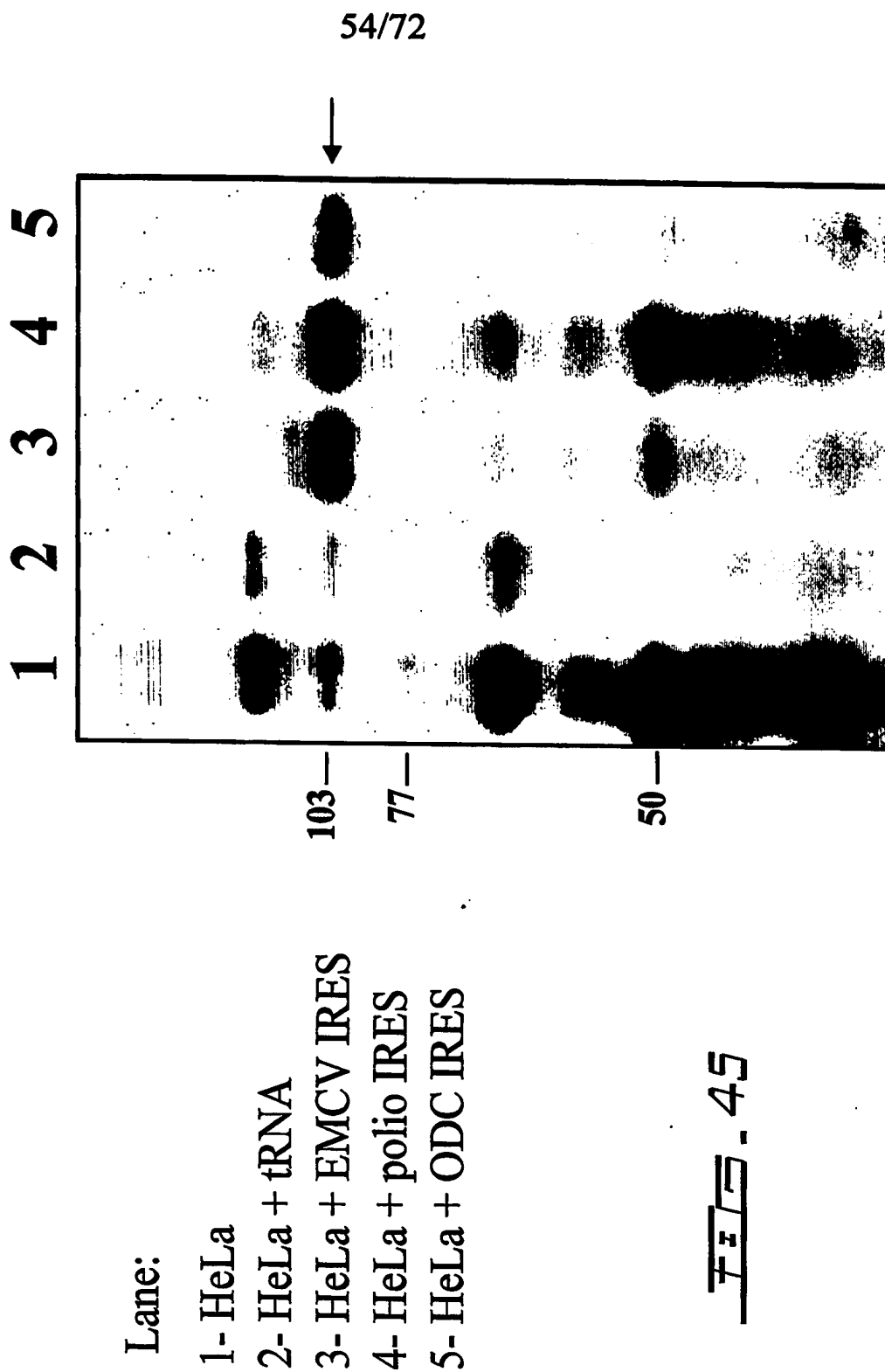
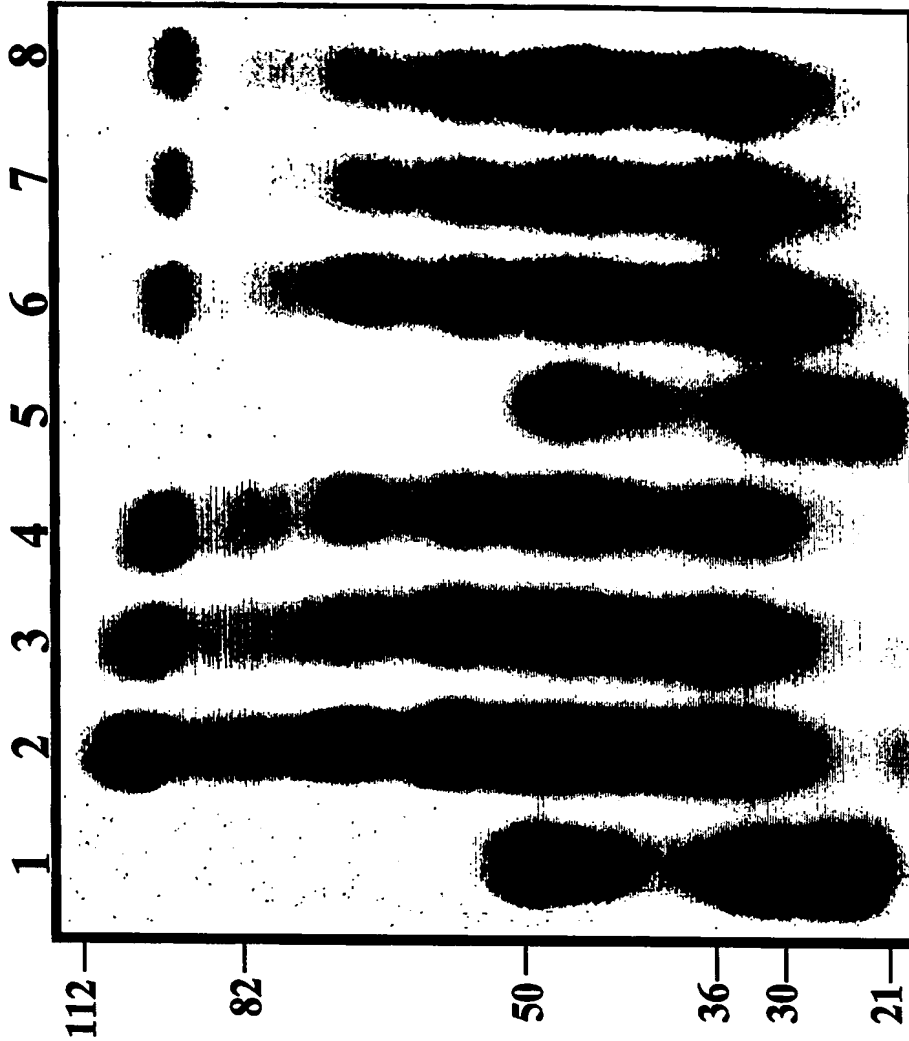


FIG. 45

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Lane:

1- PBMCs NT

2- PBMCs treatment 1

3- PBMCs treatment 2

4- PBMCs treatment

2+DEVA

5- PBMCs NT + HIV

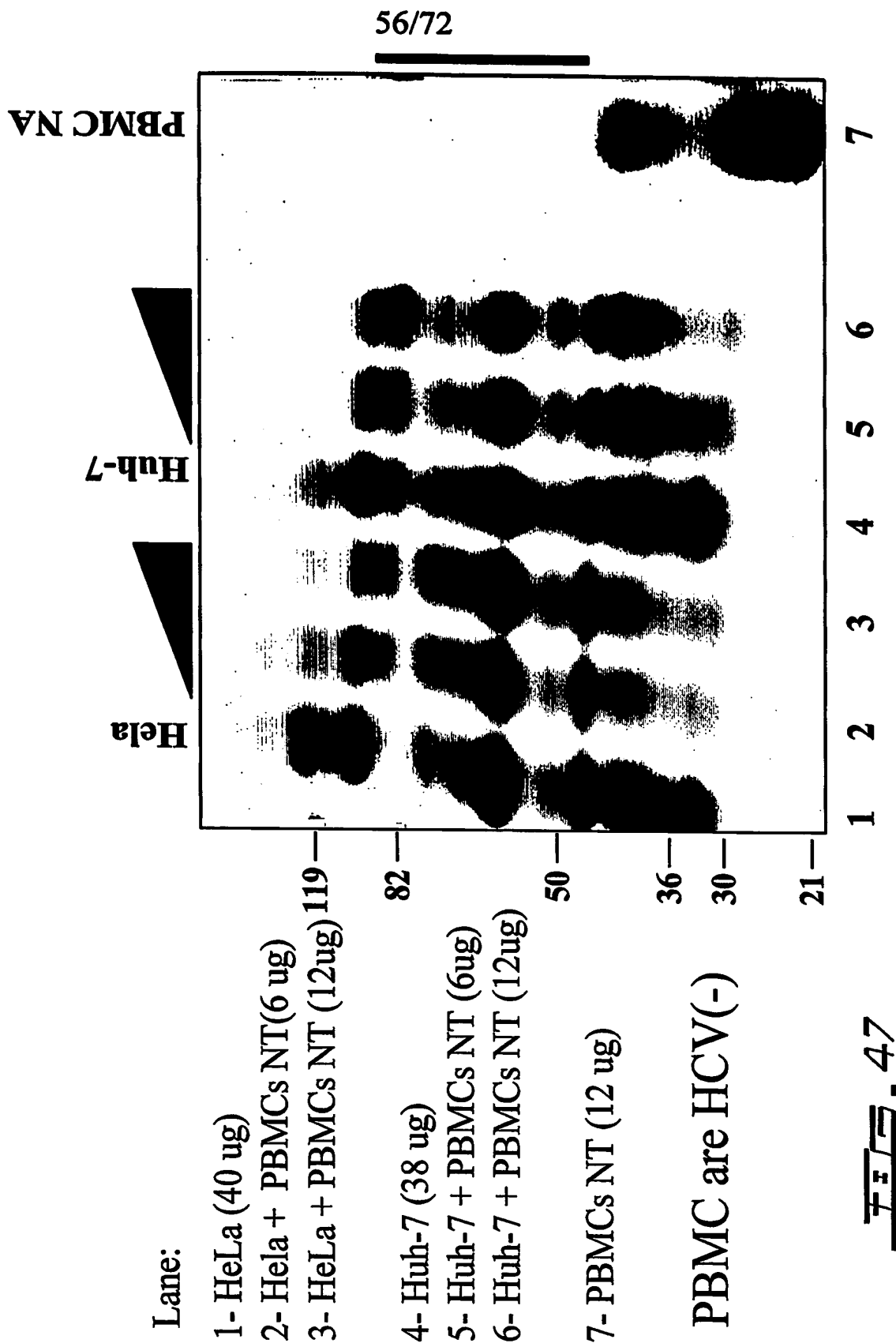
6- PBMCs treatment 1 + HIV

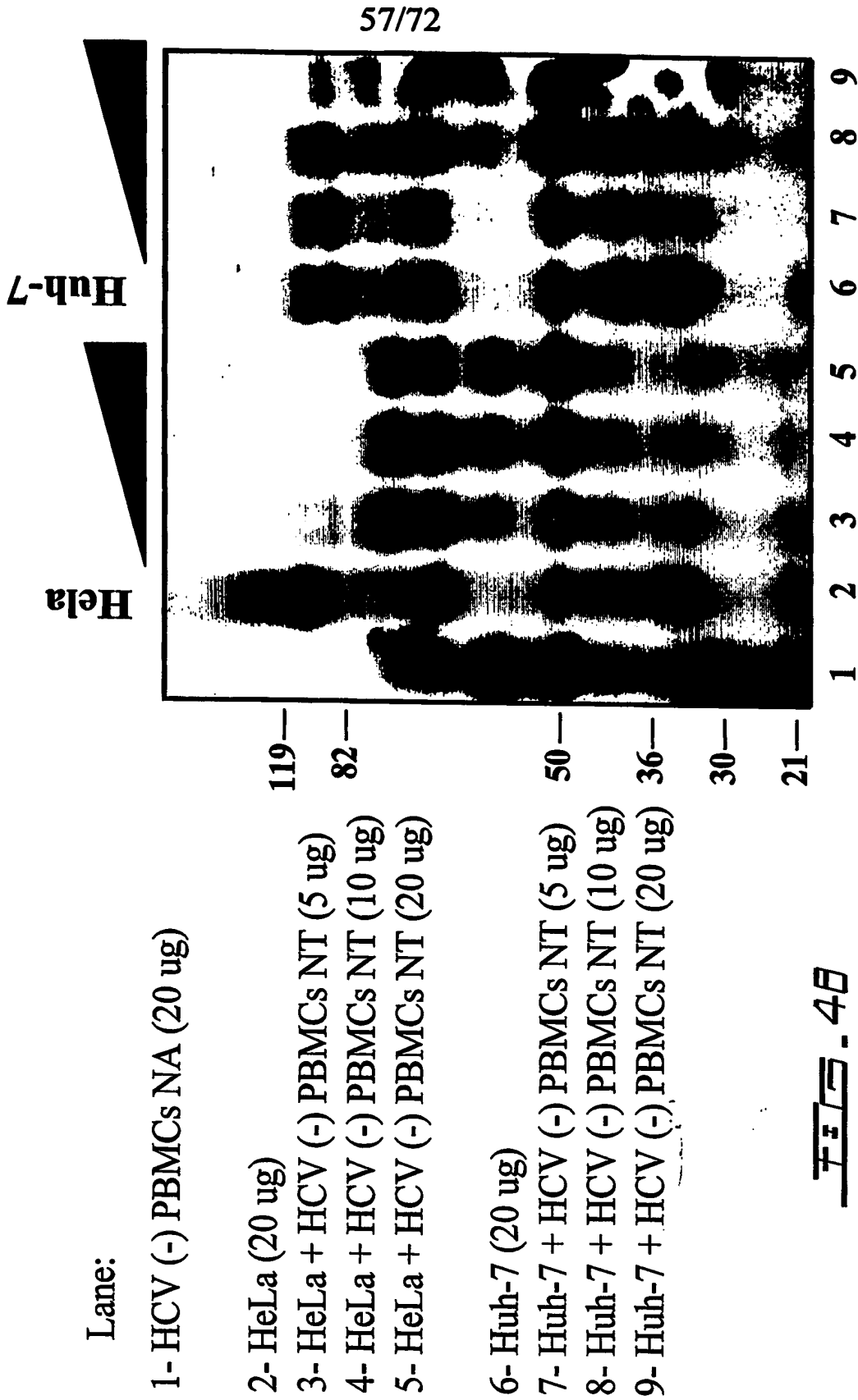
7- PBMCs treatment 2 + HIV

8- PBMCs treatment 2

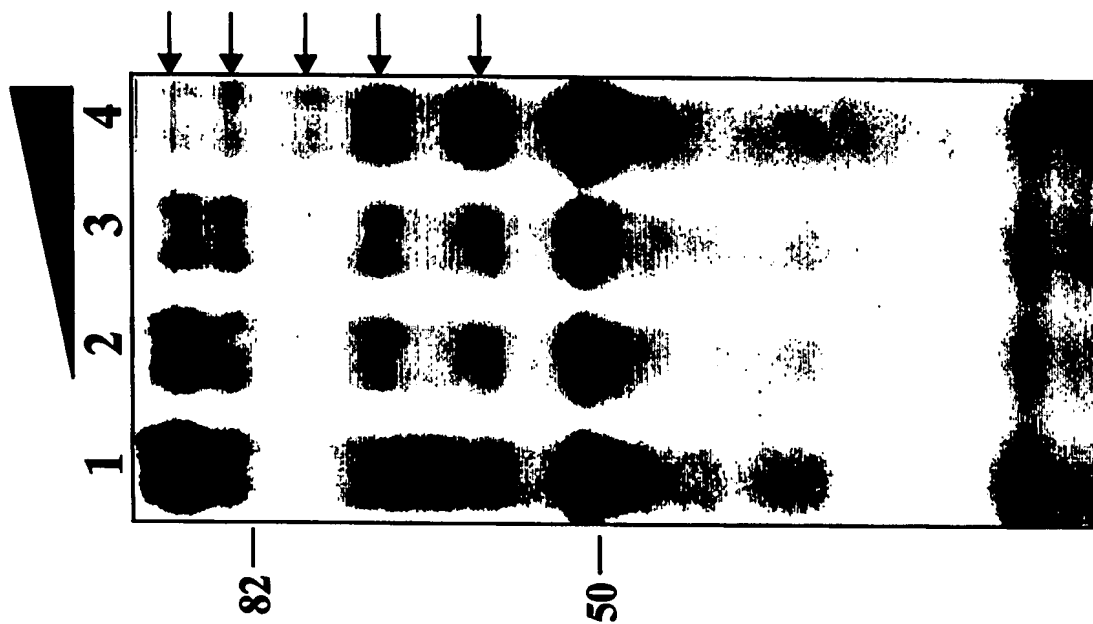
+DEVA+HIV

7575.45





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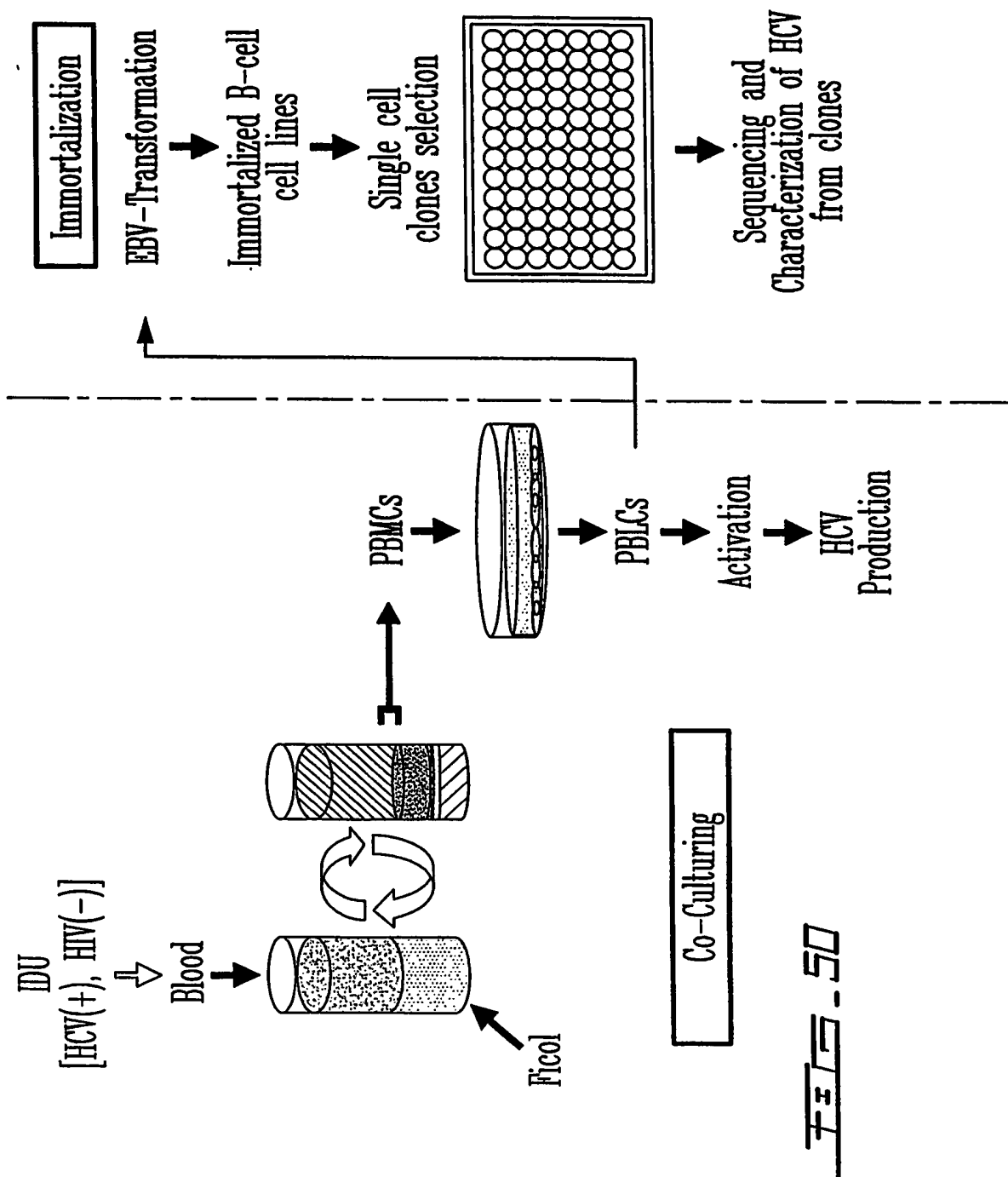


Lane:

- 1- Huh-7 (20ug)
- 2- Huh-7 + HCV (-) PBMCs NT (5ug)
- 3- Huh-7 + HCV (-) PBMCs NT (10ug)
- 4- Huh-7 + HCV (-) PBMCs NT (20ug)

FIG. 49

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HCV(+)- EBV-Transformed B-Cells.

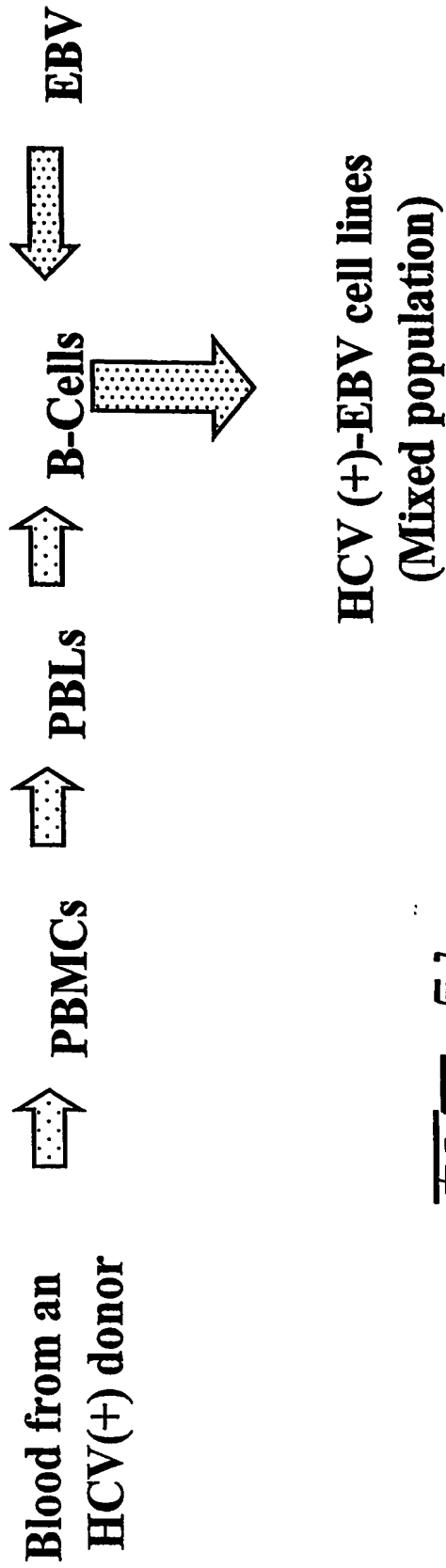


FIG. 51

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HCV RNA is detected in mixed population of EBV-transformed B-cells

HCV (+) Strand RNA

Cell line	Non-Stimulated cells RNA Copies /10 ⁶ cells	Stimulated cells RNA Copies /10 ⁶ cells
EBV-1	4.66x10 ⁵	2.33x10 ⁶
EBV-2	2.77x10 ⁵	7.91x10 ⁴
EBV-3	3.96x10 ⁶	4.02x10 ⁵
EBV-4	2.03x10 ⁶	1.57x10 ⁶
EBV-6	1.41x10 ⁶	4.32x10 ⁵
EBV-HCV (-)	0	0

GAPDH mRNA

Cell line	Non-Stimulated cells RNA Copies /10 ⁶ cells	Stimulated cells RNA Copies /10 ⁶ cells
EBV-1	2.23x10 ⁸	2.19x10 ⁸
EBV-2	8.73x10 ⁸	2.25x10 ⁸
EBV-3	1.83x10 ⁹	1.77x10 ⁹
EBV-4	5.48x10 ⁸	3.79x10 ⁸
EBV-6	1.26x10 ⁹	9.42x10 ⁸
EBV-HCV (-)	9.27x10 ⁷	3.62x10 ⁸

FIG - 52

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Control EBV-HCV (-); anti-Core

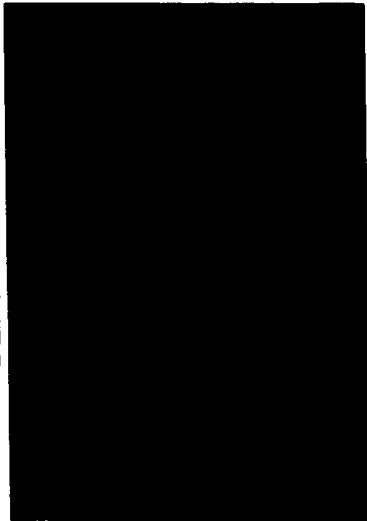
Phase



Dapi



Anti-Core



Dapi/Anti-Core



Phase/Dapi/Anti-Core

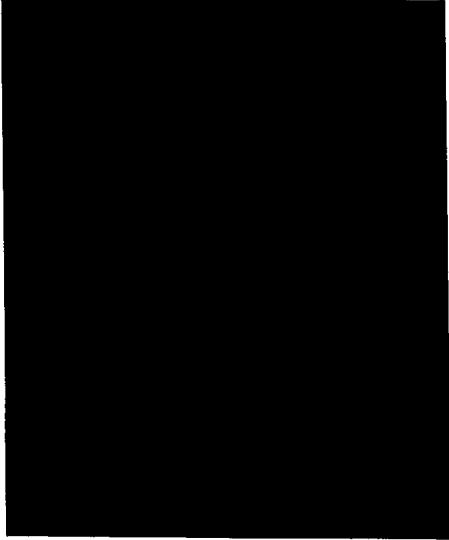


FIG. 53A

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Detection of Core in EBV-2

Anti-Core



Dapi



Phase



Phase/Dapi/Anti-Core



Dapi/Anti-Core

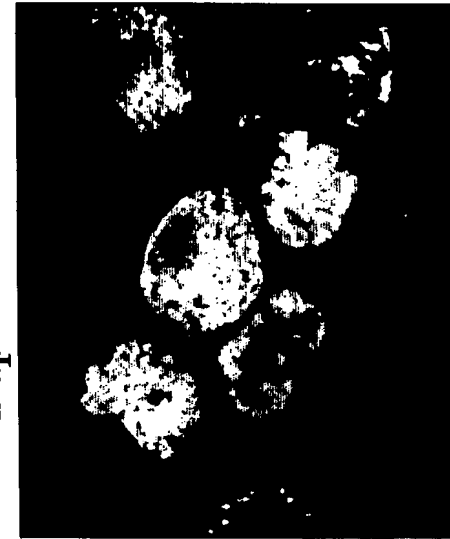


FIG. 53B

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HCV(+)-EBV-Transformed B-Cells.

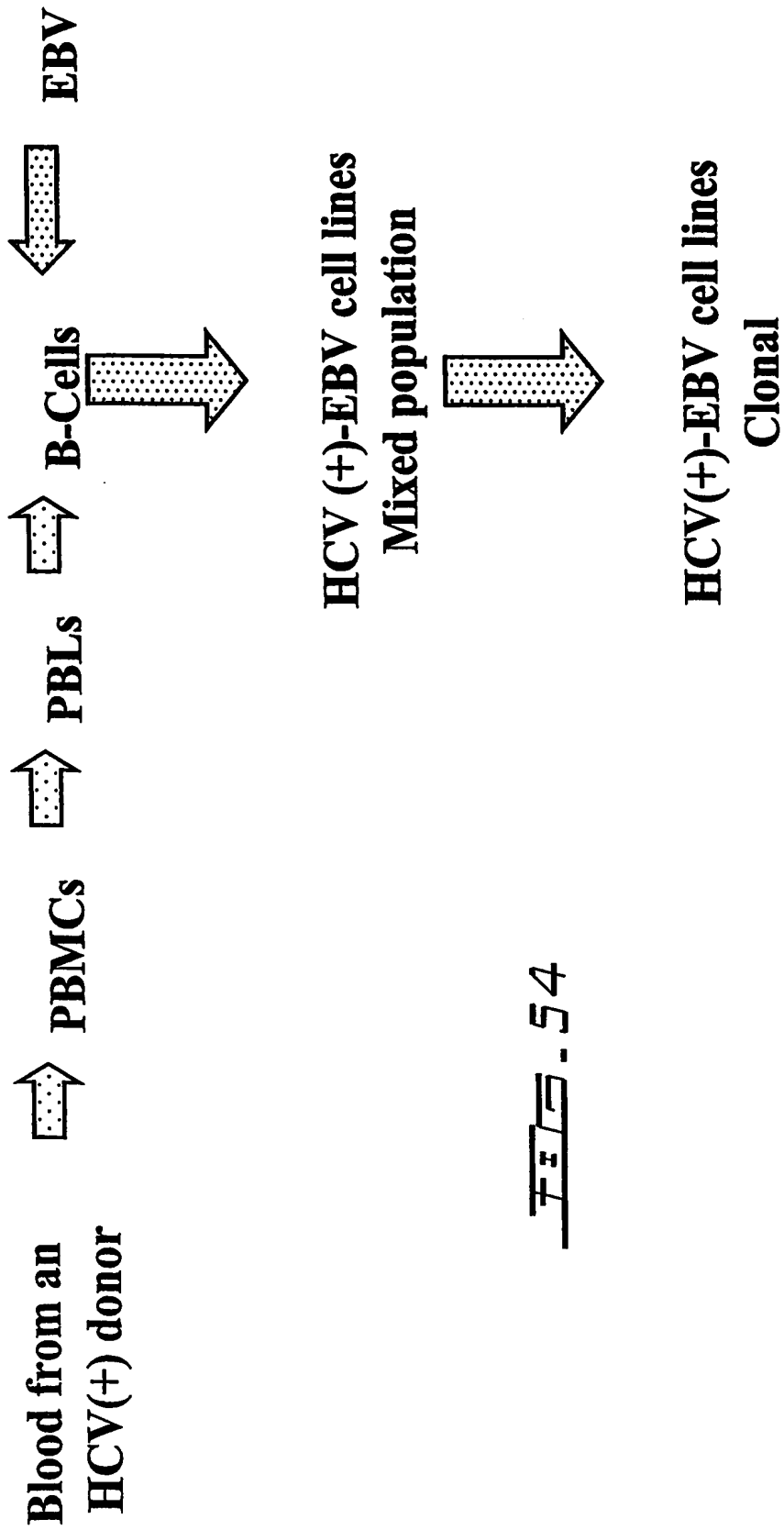
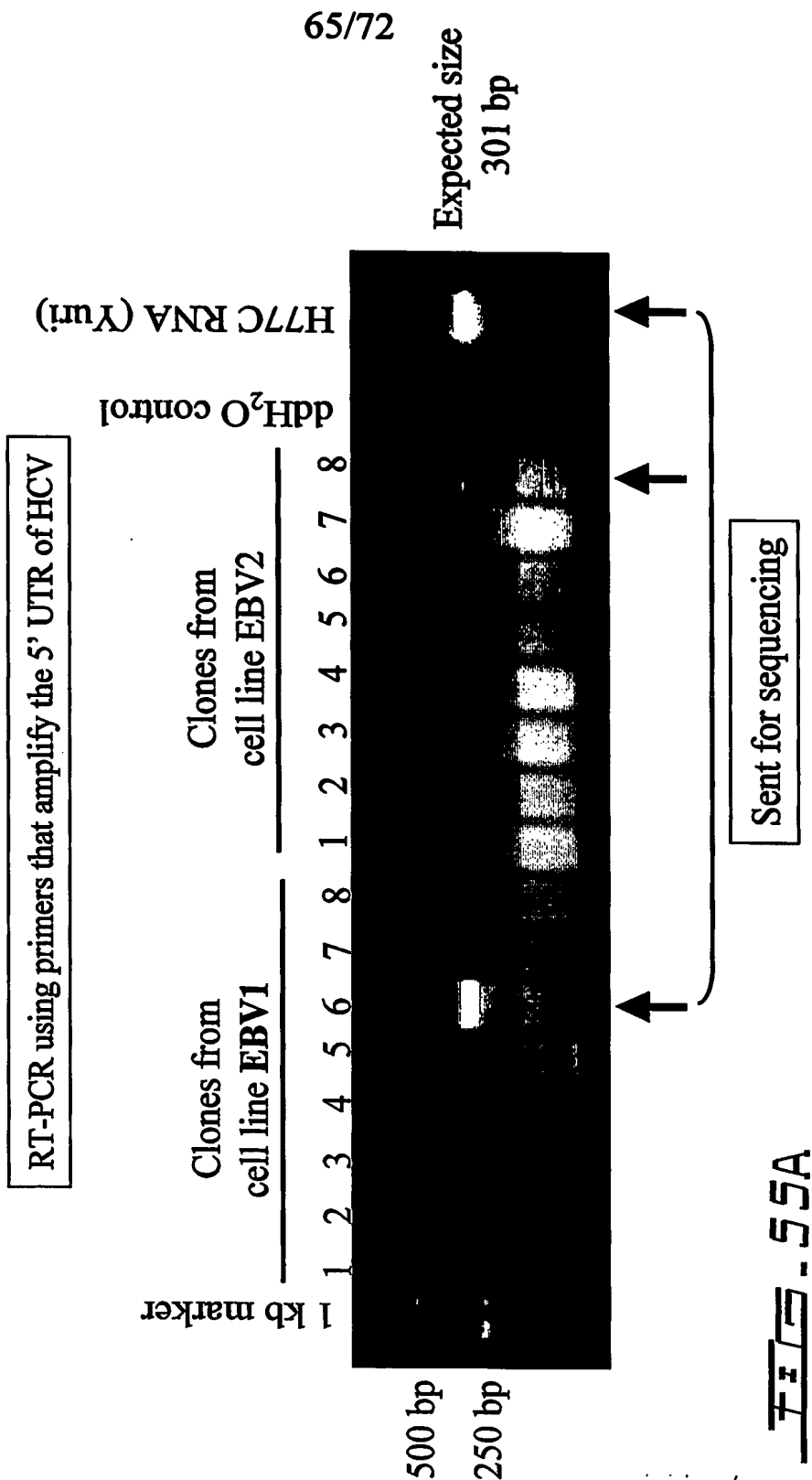


FIG. 54



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**Alignment: H77C (RT-PCR positive control) sequence (top)/
EBV1 clone 6 sequence (bottom)**

CACTCCCCTGTGAGGA	ACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGT		
CACTCCCCTGTGAGGA	ACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGT		
TAGTATGAGTGTGTCG	TGACGCCTCCAGGACCCCCCTCCCGGAGAGCCATAGTGGTC		
TAGTATGAGTGTGTCG	TGACGCCTCCAGGACCCCCCTCCCGGAGAGCCATAGTGGTC		
		<u>G</u>	
TGCGGAACCGGTGAGT	ACACCGGAATTGCCAGGACCGGGTCCTTTCTTGGATAA		
TGCGGAACCGGTGAGT	ACACCGGAATTGCCAGGACCGGGTCCTTTCTTGGATTA		
ACCCGCTCA	ATGCCCTGGAGATTGGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAG		
ACCCGCTCA	ATGCCCTGGAGATTGGGGCGTGCCCCCGCGAGACTGCTAGCCGAGTAG		
TGTTGGGTCGCGAA	AGGCCCTTGTGGTACTGCCTGATAGGGT		
TGTTGGGTCGCGAA	AGGCCCTTGTGGTACTGCCTGATAGGGT		

File 55B

Blue: sequence from virus in the serum (MLL-005).

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Alignment: H77C (RT-PCR positive control) sequence (top)/
EBV2 clone 8 sequence (bottom).

CCAGGACCCCCCTCCCGGAGAGCCATAGTGGTCTGCGGAACC
CCAGGACCCCCCTCCCGGAGAGCCATAGTGGTCTGCGGAACC

GGTGATACACCGGAATTGCCAGGACGCCGGTCTTCTTGG
GGTGATACACCGGAATTGCCAGGACGCCGGTCTTCTTGG

ATAAACCCGCTCAATGCCCTGGAGATTGGGCGTGCCCCCGCAAG
ATAAAZCCGCTCAATGCCCTGGAGATTGGGCGTGCCCCCGCAAG

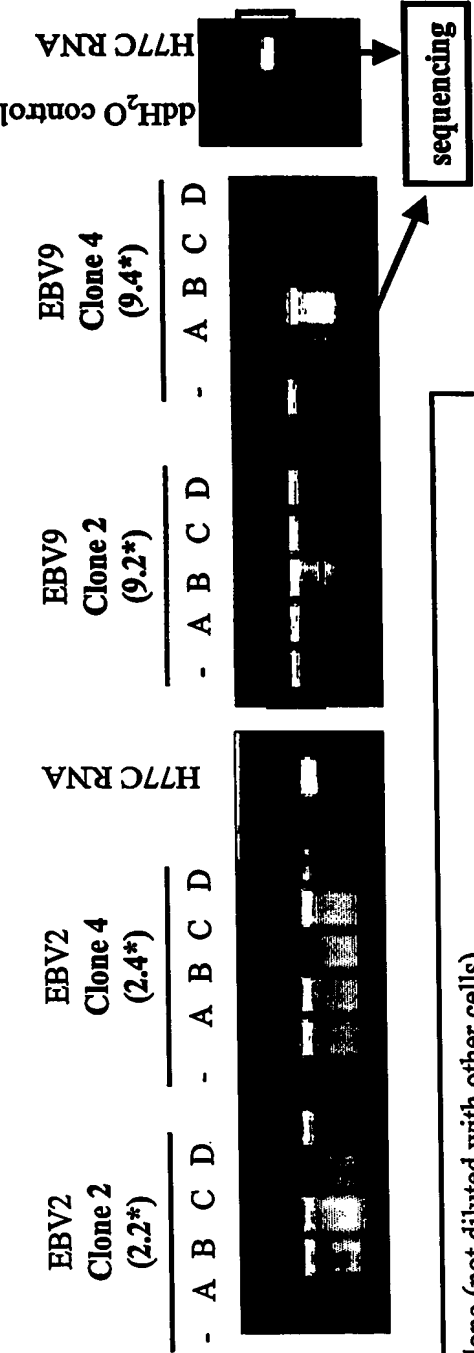
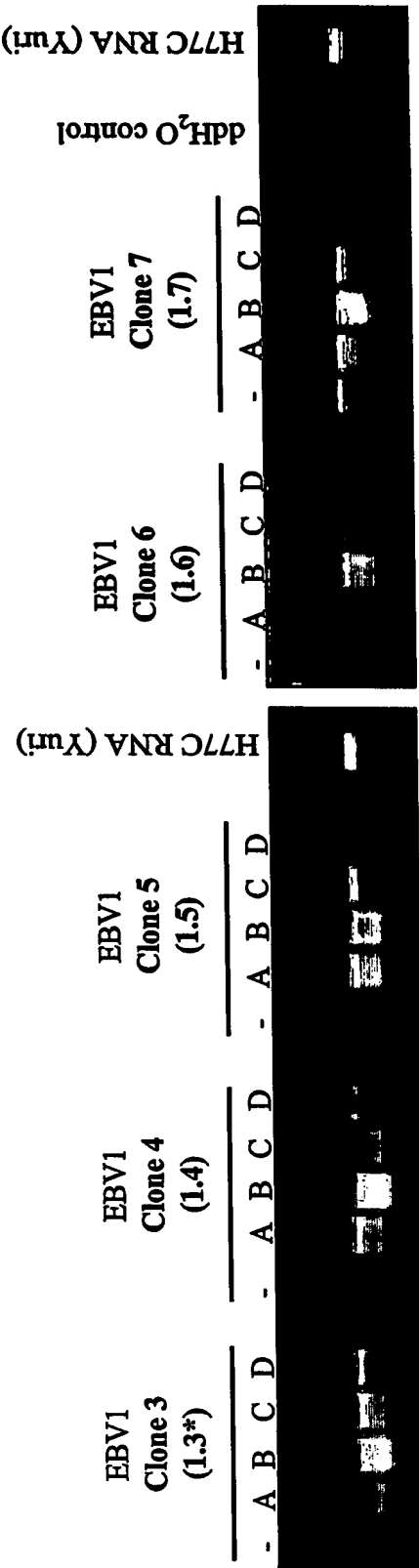
ACTGCTAGCCGAGTAGTGTGGGTGCGAAAGGCCTTGTGGTAC
ACTGCTAGCCGAGTAGTGTGGGTGCGAAAGGCCTTGTGGTAC

TGCCGTAGGGTGCTTGCGAGTGCCCCGGAGGTCTCGTAGAC
TGCCGTAGGGTGCTTGCGAGTGCZCCGGGAGGTCTCGTAGAC

CGTGCA
CGTGCA

FIG. 55C

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- = clone alone (not diluted with other cells)
A = diluted 1:10 with MT4 cell line (HTLV1 transformed T cells)
B = diluted 1:10 with BJAB cell line (ATCC non-EBV transformed B cells)
C = diluted 1:10 with HLA 006 cell line (EBV transformed HCV- PBLs)
D = diluted 1:10 with JAM cell line (EBV transformed HCV- PBLs)

FIG-56

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Alignment of all 9.2 sequences

H77C CACTCCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT
 9.2 final seq CACTCCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT
 9.2a final seq CACTCCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT
 9.2b final seq CACTCCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT
 9.2c final seq CACTCCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT
 9.2d final seq CACTCCCCCTGTGAGGAACTACTGTCTTACGCAGAAAGCGTCT

 H77C AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC
 9.2 final seq AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC
 9.2a final seq AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC
 9.2b final seq AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC
 9.2c final seq AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC
 9.2d final seq AGCCATGGCGTTAGTATGAGTGTCTGTCAGCCTCCAGGACCCCC

 H77C CCTCCCGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC
 9.2 final seq CCTCCCGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC
 9.2a final seq CCTCCCGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC
 9.2b final seq CCTCCCGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC
 9.2c final seq CCTCCCGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC
 9.2d final seq CCTCCCGGAGAGCCATAGTGGTCTGCGGAACCGGTGAGTACAC

- = clone alone (not diluted with other cells)

a= diluted 1:10 with MT4 cell line (HTLV1 transformed T cells)

b= diluted 1:10 with BJAB cell line (ATCC non-EBV transformed B cells)

c= diluted 1:10 with HLA 006 cell line (EBV transformed HCV- PBLs)

d= diluted 1:10 with JAM cell line (EBV transformed HCV- PBLs)

Red= Variation with respect to clone 9.2

FIG. 57A

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Alignment of all 9.2 sequences

H77C	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCT
9.2 final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCT
9.2a final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATTAACCCGCT
9.2b final seq	CGGAATTGCCGGGAAGAC <u>T</u> GGGTCCTTTCTTGGATAAACCC <u>A</u> CT
9.2c final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATAAACCCGCT
9.2d final seq	CGGAATTGCCAGGACGACCGGGTCCTTTCTTGGATTAATCCGCT
H77C	CAATGCCTGGAGATTGTGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2 final seq	CAATGCCTGGAGATTGTGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2a final seq	CAATGCCTGGAGATTGTGGCGTGCCCCCGCGAGACTGCTAGCCG
9.2b final seq	C <u>T</u> ATGCCCGG <u>CC</u> CA <u>T</u> TTGGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2c final seq	CAATGCCTGGAGATTGTGGCGTGCCCCCGCAAGACTGCTAGCCG
9.2d final seq	CAATGCCTGGAGATTGTGGCGTGCCCCCGCGAGACTGCTAGCCG

FIG. 57B

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Alignment of all 9.2 sequences

H77C	AGTAGTGTGGGTCGCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2 final seq	AGTAGTGTGGGTCGCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2a final seq	AGTAGTGTGGGTCGCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2b final seq	AGTAGCCTGGGTTGCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2c final seq	AGTAGTGTGGGTCGCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
9.2d final seq	AGTAGTGTGGGTCGCGAAAGGCCCTTGTGGTACTGCCCTGATAGG
H77C	GTGCTTGCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCA
9.2 final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCA
9.2a final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCA
9.2b final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCA
9.2c final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCA
9.2d final seq	GTGCTTGCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCA

Fig. 57B (Cont.)

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**Variation found when clone 9.2
is diluted in BJAB cells (---)
or in EBV-Näive cells (-----)**

